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Bacterial symbionts in human blood-feeding arthropods: Patterns, general mechanisms and effects of global ecological changes

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ABSTRACT

Due to their high impact on public health, human blood-feeding arthropods are one of the most relevant animal groups. Bacterial symbionts have been long known to play a role in the metabolism, and reproduction of these arthropod vectors. Nowadays, we have a more complete picture of their functions, acknowledging the wide influence of bacterial symbionts on processes ranging from the immune response of the arthropod host to the possible establishment of pathogens and parasites. One or two primary symbiont species have been found to co-evolve along with their host in each taxon (being ticks an exception), leading to various kinds of symbiosis, mostly mutualistic in nature. Moreover, several secondary symbiont species are shared by all arthropod groups. With respect to gut microbiota, several bacterial symbionts genera are hosted in common, indicating that these bacterial groups are prone to invade several hematophagous arthropod species feeding on humans. The main mechanisms underlying bacterium-arthropod symbiosis are discussed, highlighting that even primary symbionts elicit an immune response from the host. Bacterial groups in the gut microbiota play a key role in immune homeostasis, and in some cases symbiont bacteria could be competing directly or indirectly with pathogens and parasites. Finally, the effects climate change, great human migrations, and the increasingly frequent interactions of wild and domestic animal species are analyzed, along with their implications on microbiota alteration and their possible impacts on public health and the control of pathogens and parasites harbored in arthropod vectors of human parasites and pathogens.

1. Introduction

Arthropods that feed on human blood are composed mainly by ticks of the order Ixodida, dipterans of the families Culicidae, Glossinidae and subfamily Phlebotominae, hemipterans of the family Cimicidae and the subfamily Triatominae, and phthirapterans of the families Pediculidae and Pthiridae (Lehane, 2005). These human blood-feeding arthropods (HBFA) have been in the focus of attention worldwide, being vectors for pathogens and parasites directly or indirectly affecting millions of people; such is the case of diseases regarded as neglected or poverty-linked, which currently are top priorities for the World Health Organization (WHO) and organizations like the Drugs for Neglected Diseases initiative (DNDi), Médecins sans frontières (MSF), and most Health Ministries in affected countries (Sixty-Third World Health Assembly A63/17; Resolution CD55-R9-s 2016; Resolution CD55-15-s, item 15; Benelli and Duggan, 2018).

Symbiotic bacteria have been fundamental in the survival of hematophagous arthropods. Classically, these bacteria were formerly known to participate in the biosynthesis of B-complex vitamins (Rio et al., 2016). Now we know that they are key in the homeostasis of the peculiar arthropod immune system (IS), which has been described in recent years (Baxter et al., 2017; Eleftherianos et al., 2013; Garcia et al., 2010). Finally, symbiotic bacteria are actively involved in the establishment and/or reproduction of pathogens or parasites for which hematophagous arthropods can be vectors (Azambuja et al., 2005; Weiss and Aksoy, 2011).

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Table 1
Bloodsucking arthropod feedings on humans, pathogen and/or parasite that transmitted and human disease. B = Bacteria, V = Virus, P = Protoza, N = Nematode.

Taxa	Representative species or genera	Pathogen or Parasite transmitted	Human disease
Chelicerata			
Arachnida			
Ixodidae	*Amblyoma americanum*	*Ehrlichia chaffeensis*[B]	Human monocytotropic ehrlichiosis.
		Francisella tularensis[B]	Tularemia (Jongejan and Uilenberg, 2004)
	Amblyoma cajennense	*Rickettsia rickettsii*[B]	Rocky mountain spotted fever (Jongejan and Uilenberg, 2004)
	Amblyomma maculatum	*Rickettsia parkeri*[B]	American tick bite fever (Florin et al., 2013)
	Amblyomma variegatum	*Rickettsia africae*[B]	African tick-bite fever (Parola et al., 1999)
	Dermacentor andersoni	*Rickettsia rickettsii*[B]	Rocky mountain spoted fever (Jongejan and Uilenberg, 2004)
		Coltivirus[V]	Colorado tick fever (Sonenshine and Roe, 2014)
	Dermacentor variabilis	*Rickettsia rickettsii*[B]	Rocky Mountain Spoted fever (Jongejan and Uilenberg, 2004)
	Dermacentor occidentalis	*Rickettsia rickettsii*[B]	Rocky Mountain Spoted fever (Sonenshine and Roe, 2014)
	Dermacentor reticulatus	*Rickettsia slovaka*[B]	Human rickettsiosis (Jongejan and Uilenberg, 2004)
	Dermacentor silvarum	*Rickettsia heilongjiangensis*[B]	Unnamed (de la fuente et al., 2008)
	Dermacentor marginatus	*Rickettsia slovaka*[B]	Human rickettsiosis
		Coxiella burnetii[B]	Human Q-fever (Jongejan and Uilenberg, 2004)
	Ixodes ricinus	*Borrelia spp.*[B]	Lyme disease (de la fuente et al., 2008)
		Babesia microti[P]	Human babesiosis
		Anaplasma phagocytophilum[B]	Human granulocytic ehrlichiosis (Jongejan and Uilenberg, 2004)
		Flavivirus[V]	Tick-borne encephalites (Sonenshine and Roe, 2014)
	Ixodes scapularis	*Babesia microti*[P]	Human babesiosis
		Anaplasma phagocytophilum[B]	Human granulocytic ehrlichiosis (Jongejan and Uilenberg, 2004)
		Borrelia spp.[B]	Lyme disease (de la fuente et al., 2008)
	Ixodes persulcatus	*Borrelia* spp.[B]	Lyme disease (de la fuente et al., 2008)
		Flavivirus[V]	Tick-borne encephalites (Sonenshine and Roe, 2014)
	Ixodes pacificus	*Anaplasma phagocytophilum*[B]	Human granulocytic anaplasmosis
		Borrelia spp.[B]	Lyme disease (de la fuente et al., 2008)
	Haemaphysalis longicornis	*Rickettsia japonica*[B]	Human rickettsiosis (Jongejan and Uilenberg, 2004)
	Haemaphysalis flava	*Rickettsia japonica*[B]	Human rickettsiosis (Jongejan and Uilenberg, 2004)
	Rhipicephalus sanguneus	*Rickettsia conorii*[B]	Tick bite fever in humans (Jongejan and Uilenberg, 2004)
	Hyalomma marginatum	Nairovirus[V]	Crimean-Congo haemorrhagic fever virus (Jongejan and Uilenberg, 2004)
	Ornithodoros moubata	*Borrelia duttonii*[B]	African human relapsing fever Jongejan and Uilenberg, 2004)
Insecta			
Diptera	*Aedes aegypti*	Dengue Virus[V]	Dengue (Eldridge, 2005)
Culicidae		Chikungunya Virus[V]	Chikungunya virus disease (Morrison, 2014)
		Zika Virus[V]	Zika virus disease (Enserink, 2015; Benelli and Romano, 2017)
		Yellow Fever Virus[V]	Yellow fever (Eldridge, 2005)
		Dirofilaria immitis[N]	Filariasis Eldridge, 2005)
	Aedes albopictus	Dengue Virus[N]	Dengue (Eldridge, 2005)
		Chikungunya Virus[v]	Chikungunya virus disease (Morrison, 2014)
		Yellow Fever Virus[V]	Yellow fever (Eldridge, 2005; Benelli and Romano, 2017)
		Zika Virus[V]	Zika virus disease (Enserink, 2015)
		Dirofilaria immitis[P]	Filariasis (Eldridge, 2005)
	Aedes triseriatus	La Crosse Virus[V]	La Crosse encephalitis (Eldridge, 2005)
	Anopheles spp.	*Plasmodium* spp.[P]	Malaria
		*Wuchereria bancrofti**N**, Brugia malayi*[N]	Filariasis (Mehlhorn, 2016)
		O'nyong nyong Virus[V]	O'nyong'nyong fever (Rezza et al., 2017)
		Rickettsia felis [B] (An. gambie)	Cat-flea typhus (Dieme et al., 2015)
	Culex spp.	West Nile Virus[V]	West Nile fever
		Japanese Encephalitis Virus[V]	Japanese encephalitis
		Saint Louis Encephalitis Virus	Saint Louis encephalitis
		Wuchereria bancrofti[N],	Filariasis
		Brugia malayi[N]	Avian malaria (Eldridge, 2005)
		Dirofilaria immitis[N]	
		Plasmodium sp.[P]	
	Mansonia spp.	*Wuchereria bancrofti*[N]	Filariasis (Eldridge, 2005; Mehlhorn, 2016)
		Brugia malayi[N]	
Diptera	*Phlebotomus* spp.	Bunyaviridae[V]	Sandfly fever
Psychodidae		*Leishmania* spp.[P]	Visceral and cutaneous Leishmaniasis (Moss and Beeching, 2010)
		Phlebovirus[V]	Pappataci fever (Munstermann, 2005)
	Lutzomyia spp.	*Bartonella bacilliformis*[B]	Bartonellosis
		Leishmania spp.[P]	Leishmaniasis (Moss and Beeching, 2010)
	Glossina spp.	*Trypanosoma* spp.[P]	African trypanosomiasis (Moss and Beeching, 2010)

(*continued on next page*)

Table 1 (*continued*)

Taxa	Representative species or genera	Pathogen or Parasite transmitted	Human disease
Diptera			
Glossinidae			
Hemiptera	*Rhodnius* spp.	*Trypanosoma cruzi*[P]	American trypanosomiasis or Chagas disease (Moss and Beeching, 2010; de Fuentes-Vicente et al., 2018)
Triatominae	*Dipetalogaster* spp.		
	Panstrongylus spp.		
	Triatoma spp.		
Hemiptera	No evidence that bed bugs are vector		(Lai et al., 2016; Meriweather et al., 2013)
Cimicidae	disease		
Insecta	*Pediculus h. humanus*	*Rickettsia prowazekii*[B]	Epidemic typhus
Phthiraptera		*Bartonella quintana*[B]	Trench fever
Pediculidae		*Borrelia recurrentis*[B]	Relapsing fever (Veracx and Raoult, 2012)

In this scenario, the present review is aimed to collect and analyze recent information about the various species of symbiont bacteria and their functions in HBFA acting as vectors of the main etiologic agents of neglected diseases, which cause high morbidity and mortality every year throughout the world (Table 1). The vector activity of the blood-feeding arthropods has become even more challenging for public health worldwide since several species recently showed a wider distribution range, due to global climate change, massive migration routes, and other factors (Epstein, 2001; Benelli et al., 2018). For instance, an increase in the number of deaths caused by vector-borne diseases in the last 100 years has been recently reported in United States of America (Hansen et al., 2016). Analyzing symbiont bacteria in HBFA will deepen our understanding on the role of symbionts in their hosts, including their impact on the arthropod immune response and their influence on the establishment, reproduction, and transmission of pathogens or parasites, as well as to identify bacterial species that could be used as biological control agents of HBFA or pathogens and/or parasites for which the former are vectors.

Herein, a distinction was made between pathogens and parasites. Pathogens are defined as any microorganism like viruses and bacteria, which exhibit higher reproduction rates than parasites. On the other hand, parasites are animal groups like nematodes and platyhelminths, fungi, and protozoa (eukaryotic organisms) that exhibit lower reproductive rates than pathogens (Loker and Hofkin, 2015). Finally, there are differences in the way to take the nutrients from the hosts between pathogens and parasites.

2. Classification of bacterial symbiosis in arthropods

In their evolutionary history, bacteria and arthropods have established a wide variety of symbiotic relationships (Heddi and Gross, 2012; Nation, 2016). Most of these bacterial species are found as commensal or mutualistic organisms within arthropods (Douglas, 2015; Heddi and Gross, 2012). The functions of mutualist bacteria in insects are related to aspects like decomposing complex compounds (e.g., lignin and wood), amino acid and vitamin synthesis, and reproduction (Brownlie and Johnson, 2009; Douglas, 2015).

Recently, symbiont bacteria have also been reported to have a crucial role in the immune system, either as immunostimulators of certain pathways of the immune response or in processes like immune priming and as a direct defense against pathogens, parasites, and parasitoids (Eleftherianos et al., 2013). However, research documenting negative effects (e.g., pathogenicity) of several bacterial species and strains on a wide number of arthropod species also received great attention, being a specially interesting topic to control arthropod species that act as pests or as vectors for animal and plant diseases (Jurat-Fuentes and Jackson, 2012; Van Driesche et al., 2008).

When it is discussed the origin and evolution of groups of mutualist bacteria in arthropods, only symbiotic relationships that have occurred for long periods of time, generally millions of years, are usually considered as true mutualist bacteria (Douglas, 2015; Heddi and Gross, 2012). Regularly, mutualist bacteria are typically harbored in specific cells of the host (called bacteriocytes) that in some species can form grouped sets (called bacteriomes) located in the gut, hemocoel, and fat body of insects, or in salivary glands, eggs, ovaries, gut, and Malpighian tubules of ticks (Narasimhan and Fikrig, 2015; Simpson and Douglas, 2013). This group of mutualist bacteria has been termed as primary symbionts (PS) whose prevalence is usually high yet variable (e.g. Verspoor and Haddrill, 2011), and have vital functions in the physiology or reproduction of their hosts. Thanks to this close coevolutionary process with their arthropod host, mutualist bacteria exhibit a vertical (usually mother-to-offspring) transmission mode, which ensures their establishment in the next generation (Douglas, 2015; Heddi and Gross, 2012).

On the other hand, commensal bacteria are regarded as secondary symbionts (SS), being species that do not fulfill a vital function within their hosts, do not infect a particular type of cell, usually have a horizontal or mixed transmission mode, and their prevalence (i.e., percentage of infected individuals) in host populations is lower than that of PS (Nation, 2016; Vavre and Braig, 2012).

While significant progress in our understanding of bacteria-arthropod symbiosis has been achieved in recent years, there are still numerous unanswered questions about such interaction. For instance, does a mutualist or commensal bacterium continue eliciting an immune response in the host? Could a bacterial species have a mutualistic behavior in some hosts and a pathogenic behavior in others? Could a symbiont bacterium in HBFA be pathogenic if it entered the human body? In response to those questions and to the increasingly comprehensive data from microbiota analysis in various animal taxa, the rigid traditional classification of some bacterial species in arthropod hosts as strictly pathogenic or strictly mutualistic have been modified in recent years (Brownlie and Johnson, 2009; Mushegian and Ebert, 2016; Zug and Hammerstein, 2015).

In addition, sometimes it may be difficult to classify a given species as strict PS or SS, since some strains or life cycle stages may lack one or more of the characteristics mentioned above. An illustrative example of this duality is the genus *Wolbachia* (α-Proteobacteria: Rickettsiales), which a few years ago was classified as an intracellular parasite, with negative effects in one or more fitness-related traits of almost all arthropod (chelicerates, insects, and crustaceans) and nematode species where its presence had been reported (Werren et al., 2008). However, now we know that the coevolutive processes of *Wolbachia* in each arthropod taxon may have led some strains to an obligate mutualism (Zug and Hammerstein, 2015). A major research challenge for future research will be to determine under what circumstances a species or strain could have negative, positive, or neutral effects on its host. In this review, we considered the bacterial species that are infecting intracellularly and whose transmission mode is usually vertical as PS, even if their function may not be completely mutualistic, while the rest of symbiont bacteria are categorized as SS.

Table 2
Primary and secondary bacterial symbionts in bloodsucking arthropod vectors feedings on humans. Different symbols were used to cross-link literature findings with selected references. Similar symbols are used to match their corresponding references.

Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
Chelicerata **Arachnida** **Ixodida** **(ticks)**	*Amblyomma maculatum*	*Rickettsia parkeri* *Rickettsia amblyommii,* *Rickettsia endosymbiont* *Wolbachia* sp. *Francisella* sp.	*Alistipes* sp. *Bacillus* sp. *Corynebacterium* sp. *Diaphorpbacter* sp. *Escherichia* sp. *Ewingella* sp. *Klebsiella* sp. *Lactobacillus* sp. *Micrococcus* sp. *Propionibacterium* sp. *Pseudomonas* sp. *Raoultella* sp. *Ruminococcus* sp. *Selenomonas* sp. *Shigella* sp. *Staphylococcus* sp. *Tannerella* sp. *Trabulsiella* sp.	Culture-independent methods. Pyrosequencing of the bacterial 16S rRNA. Saliva, midgut. Adults.	Budachetri et al., 2014
	Amblyomma variegatum	*Rickettsia* sp. *Coxiella* sp.		Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	Duron et al., 2017
	Amblyomma americanum	**Rickettsia amblyommii* **Arsenophonus* sp. ◊*Coxiella* sp. ●*Wolbachia* sp. □*Midichloria* sp. □*Francisella* sp.	**Acinetobacter calcoaceticus* **Brevundimonas bullata* **Citrobacter freundii* **Collimonas* sp. **Comamonas* sp. **Enterobacter* sp. **Hafnia alvei* **Klebsiella oxytoca* **Ochrobactrum* sp. **Pantoea agglomerans* **Pseudomonas* sp. **Serratia grimesii* **Wautersiella falsenii* □*Bacillus* sp. □*Ehrlichia* sp. □*Borrelia* sp. □Burkholderiaceae □Enterobacteriaceae	*Culture-independent methods. DNA sequencing and PCR-based approaches. Adults. ◊Culture-independent methods. 16S rRNA gene sequencing and diagnostic PCR. Ovaries and salivary glands. Adults and nymphs. ●Culture-independent methods. *Wolbachia*-specific 16S primers. Entire organisms. Adults and nymphs. □Culture-independent methods. Pyrosequencing of variable regions 5–3 of the bacterial rrs. Entire organisms. Adults and nymphs.	*Clay et al., 2008; ◊ Klyachko et al., 2007 ●Zhang et al.,2010 □Williams- Newkirk, et al., 2014
	Amblyomma cajennense	*Coxiella* sp. *Francisella* sp. *Rickettsia* sp.		Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	Duron et al., 2017
	Dermacentor andersoni	**Rickettsia peacokii* ◊*Arsenophonus* sp. ●*Francisella* sp.	●*Acinetobacter* sp.	*Culture-independent methods. PCR assay with primer sets derived from eubacterial 16 s ribosomal DNA. Tick tissue infected. Adults. ◊Culture-independent methods. DNA sequencing. Entire organisms. Adults. ●Culture-independent methods. High-throughput sequence analysis. Midgut and salivary glands. Adults.	*Niebylski et al., 1997 ◊Dergousoff and Chilton, 2010 ●Clayton et al., 2015
	Dermacentor variabilis	**Arsenophonus* sp. ◊*Francisella* sp. ●*Rickettsia belli* ●*Rickettsia montanensis* ●*Rickettsia rickettsii* □*Coxiella* sp.	□Clostridiales □Ruminococcaceae □Planococcaceae □*Persicirhabdus* sp. □Lachnospiraceae	*Culture-independent methods. Eubacterial 16S and rDNA specific primers. Entire organisms. Adults. ◊Culture-independent methods. Eubacterial 16S. rDNA specific Primers. Entire organisms. Adults. ●Culture-independent methods. Semi-nested PCR. Hemolymph.	*Grindle et al., 2003 ◊Sun et al., 2000 ●Carmichael and Fuerst, 2006 □Clow et al., 2018

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Table 2 (*continued*)

Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			□*Treponema* sp. □*Borrelia* sp.	□Culture-independent methods. Next generation sequencing. Entire organisms. Adults and nymphs.	
	Dermacentor occidentalis	*Rickettsia* sp. *Francisella* sp.		Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	Duron et al., 2017
	Dermacentor reticulatus	**Rickettsia* sp. ◊*Francisella* sp.		*Culture-independent methods. Transcriptomic and proteomic analysis. Entire organisms. Nymphs. ◊Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	*Villar et al., 2014 ◊Duron et al., 2017
	Dermacentor silvarum	*Coxiella* sp. *Arsenophonus* sp. *Rickettsia* sp.		Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	Duron et al., 2017
	Dermacentor marginatus	**Rickettsia* sp. ◊*Coxiella* sp. ◊*Spiroplasma* sp.	**Flavobacterium* sp. **Escherichia coli*, **Curvibacter* sp. **Acidovorax* sp. **Shigella* sp. **Stenotrophomonas maltophilia* *Bacteroidaceae	*Culture-independent methods. Microbiome Pyrosquencing. Entire organisms. Adults. ◊Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	*Tekin et al., 2017 ◊Duron et al., 2017
	Ixodes ricinus	**Rickettsia* sp. **Wolbachia* sp. **Rickettsiella* sp. ◊*Coxiella* sp. ◊*Midichloria* sp. ◊*Spiroplasma* sp.	**Bartonella* sp. **Brucella* sp. **Bordetella* sp. ** Burkholderia* sp. **Mycobacterium* sp. **Borrelia* sp. **Frankia* sp. **Clostridium* sp. **Streptomyces* sp. **Magnetospirillum* sp. **Sorangium* sp. **Bacillus* sp. **Methylobacterium* sp. **Bdellovibrio* sp. **Rhizobium* sp. **Pseudomonas* sp. **Rhodococcus* sp. **Erwinia* sp. ◊*Stenotrophomonas* sp.	*Culture-independent methods. 454- and Illumina based metagenomic. Nymphs and adults. ◊Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	*Carpi et al., 2011 ◊Duron et al., 2017
	Ixodes scapularis	◊*Rickettsia* sp. ◊*Francisella* sp. ◊*Cardinium* sp. ●*Wolbachia* sp.	**Thioclava* sp. **Delftia* sp. **Aquabacterium* sp. **Brevibacterium* sp. **Novosphingobium* sp. **Acidovorax* sp. **Comamonas* sp. **Chryseobacterium* sp. **Lactobacillus* sp. **Paenibacillus* sp. ◊*Pseudomonas* sp. ●*Acinetobacter* sp. ●*Afipia* sp. ●*Burkholderia* sp. ●*Escherichia coli* ●*Propionibacterium* sp. ●*Streptococcus* sp. ●*Stenotrophomonas* sp.	*Culture-independent methods. Pyrosequencing barcoded amplified bacterial 16S rDNA. Nymphs. ◊Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults. ●Culture-independent methods. PCR-amplified bacterial 16S rRNA genes. Entire organisms. Nymphs	*Benson et al., 2004 ◊Duron et al., 2017 ●Narasimhan et al., 2014

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Table 2 (*continued*)

Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
	Ixodes persulcatus	**Rickettsia* sp. ◊*Lariskella* sp.	**Acinetobacter* sp. **Borrelia* sp. **Brevundimonas* sp. **Chryseobacterium* sp. **Clostridium* sp. **Desulfotomaculum* sp. **Flavobacterium* sp. **Haemophilus* sp. **Halomonas* sp. **Janthinobacterium* sp. **Parapedobacter* sp. **Prevotella* sp. **Proteus* sp. **Pseudomonas* sp. **Psychrobacter* sp. **Shewanella* sp. **Sphingobacterium* sp. **Stenotrophomonas* sp. **Zhouia* sp.	*Culture-independent methods. 16S rRNA and Illumina sequencing- Entire organisms. Adults ◊Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	*Zhang et al., 2014 ◊Duron et al., 2017
	Ixodes pacificus	*Rickettsia* sp. *Francisella* sp. *Lariskella* sp. *Spiroplasma* sp.	*Acinetobacter* sp. *Agrobacterium* sp. *Bosea* sp. *Burkholderia* sp. *Chryseobacterium* sp. *Flavobacterium* sp. *Luteibacter* sp. *Lysinibacillus* sp. *Methylobacterium* sp. *Mycobacterium* sp. *Phyllobacterium* sp. *Pseudomonas* sp. *Sphingomonas* sp. *Streptomyces* sp.	Culture-independent methods.16S rRNA Sequencing by Illumina MiSeq. Entire organisms. Nymphs and adults.	Kwan et al., 2017
	Haemaphysalis longicornis	◊*Coxiella* sp.	**Brevibacterium* sp. **Caryophanon* sp. **Kocuria* sp. **Microbacterium* sp. **Microbacterium paraoxydans* **Staphylococcus cohnii* **Staphylococcus saprophyticus* **Staphylococcus sciuri* **Staphylococcus xylosus*	*Culture-independent methods. Morphological, biochemical, and 16S rDNA sequencing. Midgut. Adults. ◊Culture-independent methods. Primers for eubacterial 16S and 23S rRNA genes. Ovaries and Malpighian tubules. Nymphs and adults.	*Li et al., 2014 ◊Noda et al., 1997
	Haemaphysalis flava	*Rickettsia* sp. *Coxiella* sp.	*Acinetobacter* sp. *Corynebacterium* sp. *Cupriavidus* sp. *Citrobacter* sp. *Ehrlichia* sp. *Escherichia* sp. *Pseudomonas* sp. *Psychrobacter* sp.	Culture-independent methods. Illumina MiSeq sequencing platform for 16S rDNA amplicon analysis. Entire organisms. Egg, larvae, nymph and adults.	Duan and Cheng, 2017

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Table 2 (*continued*)

Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
	Rhipicephalus sanguneus	*Coxiella* sp. *Rickettsia* sp.	*Acinetobacter* sp. *Arthrobacter* sp. *Bhargavaea* sp. *Bradyrhizobium* sp. *Bacillus* sp. *Enterococcus* sp. *Lactobacillus* sp. *Lysinibacillus* sp. *Macrococcus* sp. *Massilia* sp. *Microbacterium* sp. *Micrococcus* sp. *Proteus* sp. *Pseudomonas* sp. *Rhodococcus* sp. *Staphylococcus* sp.	Culture-independent methods. 16S rDNA analysis on the Illumina Miseq Platform. Entire organisms. Adults and nymphs.	René-Martellet et al., 2017
	Rhipicephalus annulatus	◊*Coxiella* sp. ◊*Spiroplasma* sp.	**Flavobacterium* sp. **Escherichia coli* **Curvibacter* sp. **Acidovorax* sp. **Stenoptrophomonas maltophila* **Shigella* sp. **Variovorax* sp.	*Culture-independent methods. 16S tag-encoded FLX-titanium amplicon pyrosequencing. Entire organisms. Adults ◊Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	*Tekin et al., 2017 ◊Duron et al., 2017
	Hyalomma marginatum	*Rickettsia* sp. *Francisella* sp.	*Acidovorax* sp. *Agrobacterium* sp. *Alicycliphilus* sp. *Aquabacterium* sp. *Arcicella* sp. *Bacillus* sp. *Bacteroides* sp. *Chryseobacterium* sp. *Clostridium* sp. *Curvibacter* sp. *Diaphorobacter* sp. *Escherichia* sp. *Flavobacterium* sp. *Fluviicola* sp. *Janibacter* sp. *Kurthia* sp. *Massilia* sp. *Paucibacter* sp. *Pedobacter* sp. *Pleomorphonas* sp. *Ralstonia* sp. *Rhizobium* sp. *Rhodoferax* sp. *Ruminococcus* sp. *Segetibacter* sp. *Sphingomonas* sp. *Stenotrophonas* sp. *Variovorax* sp.	Culture-independent methods. Two primer sets were used to target the V1–V3 and V6–V8 variable regions of the 16S rRNA by pyrosequencing. Entire organisms. Adults.	Keskin et al., 2017
	Ornithodoros moubata	*Rickettsia* sp. *Francisella* sp.		Culture-independent methods. Amplifying a fragment of either the 16S rRNA gene or another housekeeping gene. Entire organisms. Adults.	Duron et al., 2017

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
Insecta **Diptera** **Nematocera** **Culicidae** **(mosquitoes)**					
	Aedes aegypti		**Asaia krungthepensis* **Asaia* sp. **Bacillus* sp. **Bacillus subtilis* **Cedecea davisae* **Citrobacter freundii* **Enterobacter aerogenes* **Enterobacter* sp. **Enterococcus caccae* **Klebsiella pneumoniae* **Kluyvera ascorbata* **Pantoea agglomerans* **Pantoea* sp. **Serratia marcescens* **Serratia* sp. ◊*Burkholderiaceae* ◊*Elizabethkingia meningoseptica* ◊*Enterococcus faecalis* ◊*Pantoea stewartii* ◊*Sphingomonas* sp. •*Acinetobacter* sp. •*Pseudomonas* sp. •*Staphylococcus saprophyticus*	*Culture-dependent and culture-independent methods and amplified 16S rRNA gene. Midgut, ovary and eggs. Adults ◊Culture-dependent and culture-independent. Cloning and sequence analysis. Gut. Adults. •Culturing methods and denaturing gradient gel electrophoresis (DGGE) and sequencing of the rrs amplicons. Entire organism. Adults.	Gusmão et al., 2010 ◊Terenius et al., 2012 •Zouache et al., 2010
	Aedes albopictus	**Rickettsia* sp. ◊*Rickettsia felis* **Wolbachia pipientis*	**Asaia* sp. **Agrobacterium sp.* **Bacillus cereus* **Bacillus* sp. **Bradyrhizobium* sp. **Citrobacter* sp. **Delftia* sp. **Enterobacter* sp. **Herbaspirillum* sp. **Pantoea agglomerans* **Pseudomonas putida* **Pseudomonas* sp. **Rhizobium* sp. **Shigella flexneri* **Staphylococcus* sp. **Stenotrophomonas maltophilia* **Yokenella regensburgei* ●*Arsenicicoccus bolidensis* ●*Arthobacter albidus* ●*Bacillus pumilus* ●*Bacilus subtilis* ●*Cellulosimicrobium cellulans* ●*Curtobacterium* sp. ●*Deinococcus* sp. ●*Dermacoccus* sp. ●*Kocuria* sp.	*Culturing methods and denaturing gradient gel electrophoresis (DGGE) and sequencing of the rrs amplicons-Entire organism. Adults. ●Culturing methods and Sequencing analysis of the 16S rDNA genes–Entire organism. Adults. ◊Culture-independent methods. Quantitative real-time PCR targeting and biotin synthase gene. Abdomen, wings, legs. Adults	*Zouache et al., 2010 ●Moro et al., 2013 ◊Socolovschi et al., 2012a

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			●*Leucobacter* sp. ●*Haematobacter massiliensis* ●*Microbacterium arborescens* ●*Microbacterium esteraromaticum* ●*Microbacterium flavescens* ●*Micrococcus pumilus* ●*Neisseria* sp. ●*Planococcus* sp. ●*Pseudomonas oryzihabitans* ●*Skermanella aerolata* ●*Sphingomonas yunnanensis* ●*Staphylococcus epidermidis* ●*Staphylococcus warneri* ●*Streptomyces* sp. ●*Terrabacter* sp. ●*Xanthomonas* sp.		
	Aedes triseriatus		*Aerococcus viridians* *Bacillus cereus* *Bacillus indicus* *Bacillus firmus* *Bacillus licheniformis* *Bacillus megaterium* *Bacillus* sp. *Lysinibacillus sphaericus* *Aeromonas popoffii* *Enterobacter cloacae* *Escherichia coli* *Exiguobacterium aestuarii* *Pseudomonas flurocescens*	Dependent and culture-independent techniques and specific real-time quantitative polymerase chain reaction assay. Midgut. Larvae and adults.	Kim et al., 2015
	Anopheles gambie	□*Rickettsia felis*	**Acinetobacter* sp. **Aerococcus* sp. **Aeromonas* sp. **Agromyces* sp. **Bacillariophyta* sp. **Chlorophyta* sp. **Chryseobacterium* sp. **Cloacibacterium* sp. **Clostridium* sp. **Comamonas* sp. **Corynebacterium* sp. **Elizabethkingia* sp. **Enterobacter* sp. **Finegoldia* sp. **Fusobacterium* sp. **Gpila* sp. **Hydrogenophaga* sp. **Klebsiella* sp. **Lactobacillus* sp. **Methylocystis* sp. **Methylophilus* sp. **Novosphingobium* sp. **Pelagibacter* sp. **Porphyrobacter* sp.	*Pyrosequencing bacterial 16S rRNA fragments. Larvae, pupae and adults. ●16 S amplicon sequencing. Entire organisms. Adults. ◊Meta-taxogenomic analysis. Midguts. Adults. □Culture-independent methods. Quantitative and traditional PCR assays specific for *Rickettsia* genes. Entire organisms. Adults.	*Wang et al., 2011 ●Buck et al., 2016 ◊Boissière et al., 2012 □Socolovschi et al., 2012b

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			**Propionibacterium* sp. **Pseudomonas* sp. **Raoultella* sp. **Rhizobium* sp. **Roseomonas* sp. **Serratia* sp. **Sphingobium* sp. ** Stenotrophomonas* sp. **Thorsellia* sp. •*Alteromonadales* •*Bacillaceae* •*Bifidobacteriales* •*Caulobacterales* •*Cytophagales* •*Flavobacteriales* •*Frankiales* •*Oceanospirillales* •*Oxakibacteraceae* •*Planococcaceae* •*Rhdobacterales* •*Rhodospirallales* •*Staphylococcaceae* ◊*Asaia* sp. ◊*Burkhoderia* sp. ◊*Neisseria* sp. ◊*Rhodococcus* sp. ◊*Rhodopseudophomonas* sp. ◊*Sphingomonas* sp.		
	Anopheles albimanus		**Enterobacter* sp. **Enterobacter cloacae* **Enterobacter amnigenus* **Serratia* sp. **S. marscencens* ◊*Aphanothece cf. castagnei* ◊*Oscillatoria* sp. ◊*Phormidium* sp. ◊*Spirulina* sp.	*Culture methods and standard biochemistry analysis. Midguts. Adults ◊Culture methods. Identification using standard keys. Larvae.	*Gonzalez-Ceron et al., 2003 ◊Vázquez-Martinez et al., 2002
	Anopheles arabiensis		*Acidovorax temperans* *Anaplasma ovis* *Bacillus silvestris* *Ehrlichia* sp. *Escherichia senegalensis* *Janibacter limosus* *Mycoplasma weyonii* *Nocordia corynebacteroides* *Paenibacillus* sp. *Serratia odorifera* *Stenotrophomonas maltophilia* *Vibrio metschnikovii*	Dependent and culture-independent techniques sequence analysis of 16S rRNA genes. Midgut. Adults.	Lindh et al., 2005
	Anopheles funestus	◊*Spiroplasma* sp. •*Rickettsia felis*	**Bacillus magaterium* **Brevundimonas diminuta*	*Culture methods and biochemistry analysis. Midguts. Adults. ◊Dependent and culture-independent techniques sequence analysis of 16S rRNA genes.	

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			*Cedecea davisae *Comamonas testosteroni *Erwinia chrysanthemi * Escherichia coli *Fluvobacterium resinocorum *Gluconobacter cerinus * Klebsiella pneumoniae *Kluyvera cryocrescens *Pantoea agglomerans *Pantoea ananas *Pseudomonas mendocina *Pseudomonas stutzeri *Salmonella choleraesuis *Stenotrophomonas maltophilia	Midgut. Adults. ●Culture-independent methods. Quantitative real-time reverse transcription PCR (qRT-PCR)-Entire organisms. Larvae and adults.	*Straif et al., 1998 ◊Lindh et al., 2005 ●Mediannikov et al., 2013
	Anopheles maculipenis		*Aeromonas bivalvium* *Aeromonas punctata* *Lysinibacillus sphaericus* *Pseudomonas mendocina*	Culture methods and differential biochemical tests and 16S rRNA gene sequence analysis. Midgut. Larvae.	Djadid et al., 2011
	Anopheles culicifacies		*Acinetobacter* sp. *Aeromonas* sp. *Bacillus* sp. *Brachybacterium* sp. *Chryseobacterium* sp. *Delftia* sp. *Exiguobacterium* sp. *Kurthia* sp. *Microbacterium* sp. *Pseudomonas* sp. *Thorsellia* sp. *Staphylococcus* sp. *Variovorax* sp.	Culture methods and 16S rRNA gene sequence analysis. Midgut. Larvae and Adults.	Chavshin et al., 2014
	Anopheles sthepensi		*Agrobacterium* sp. *Agrobacterium tumefaciens* *Chryseobacterium meningosepticum* *Comamonas* sp. *Elizatehkingia meningoseptica* *Klebsiella* sp. *Pseudomonas mendocina* *Pseudomonas tolaasii* *Serratia marcescens* ◊*Aeromonas* sp. ◊*Alcaligenes* sp. ◊*Bordetella* sp. ◊*Enterobacter* sp. ◊*Enterococcus* sp. ◊*Exiguobacterium* sp. ◊*Kocuria* sp. ◊*Microbacterium* sp. ◊*Myroides* sp. ◊*Rhodococcus* sp., ◊*Shewanella* sp. ●*Bacillus pumilus* ●*Brevundimonas aurantiaca* ●*Lysinibacillus sphaericus* ●*Pantoea agglomerans*	*Dependent and culture independent techniques and 16S rRNA gene was amplified. Larvae and adults. Midgut. ◊Cell-free culture technique and analysis of a 16S ribosomal RNA Larvae and adults. Midgut. ●Culture methods and differential biochemical tests and 16S rRNA gene sequence analysis. Midgut. Larvae	*Rani et al., 2009 ◊Chavshin et al., 2012 ●Djadid et al., 2011

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			•*Pantoea stewartii* • *Sphingomonas paucimobilis* •*Rahnella aquatilis*		
	Culex quinquefasciatus	•*Wolbachia pipientis*	**Acinetobacter calcoaceticus* **Acinetobacter junii* **Acinetobacter* sp. **Aeromonas jandaei* **Bacillus thuringiensis* **Enterococcus seriolicida* **Lactococcus garvieae* **Microbacterium oxydans* **Pantoaea agglomerans* **Pseudomonas aeruginosa* **Staphylococcus epidermidis* ◊*Acinetobacter baumannii* ◊*Acinetobacter beijerinckii* ◊*Acinetobacter lwoffii* ◊*Acinetobacter pittii* ◊*Acinetobacter radioresistens* ◊*Acinetobacter schindleri* ◊*Acinetobacter soli* ◊*Aerococcus urinaeequi* ◊*Aeromonas enteropelogenes* ◊*Aeromonas hydrophila* ◊*Aeromonas ichthiosmia* ◊*Aeromonas veronii* ◊*Arthrobacter creatinolyticus* ◊*Bacillus anthracis* ◊*Bacillus aryabhattai* ◊*Bacillus cereus* ◊*Bacillus circulans* ◊*Bacillus flexus* ◊*Bacillus licheniformis* ◊*Bacillus nealsonii* ◊*Bacillus safensis* ◊*Bacillus subtilis* ◊*Citrobacter braakii* ◊*Citrobacter freundii* ◊*Delftia lacustris* ◊*Enterobacter asburiae* ◊*Enterobacter cancerogenus* ◊*Enterobacter cloacae* ◊*Enterobacter ludwigii* ◊*Enterococcus caccae* ◊*Enterococcus faecalis* ◊*Enterococcus hirae* ◊*Enterococcus silesiacus* ◊*Escherichia coli* ◊*Escherichia hermannii* ◊*Exiguobacterium aurantiacum* ◊*Exiguobacterium indicum* ◊*Exiguobacterium profundum* ◊*Janibacter melonis* ◊*Klebsiella oxytoca*	*Conventional culture technique and analysis of a 16S ribosomal RNA (rRNA) gene sequence library. Midgut. Females adults. ◊Examination of 16S ribosomal DNA amplicons from culturable microflora. Midgut. Females adults. •DNA isolation, and polymerase chain reaction amplification of three (wsp, 16S rRNA, and ftsZ) genes. Ovaries. Females Adults.	*Pidiyar et al., 2004 ◊Chandel et al., 2013 •Thongsripong et al., 2018

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			◊*Klebsiella pneumoniae* ◊*Klebsiella variicola* ◊*Kocuria carniphila* ◊*Kocuria marina* ◊*Kocuria palustris* ◊*Kytococcus schroeteri* ◊*Lactococcus lactis* ◊*Leucobacter tardus* ◊*Lysinibacillus macroides* ◊*Microbacterium arborescens* ◊*Microbacterium imperiale* ◊*Microbacterium maritypicum* ◊*Micrococcus lylae* ◊*Micrococcus yunnanensis* ◊*Morganella morganii* ◊*Pantoea anthophila* ◊*Pantoea dispersa* ◊*Proteus vulgaris* ◊*Providencia alcalifaciens* ◊*Providencia rettgeri* ◊*Pseudomonas beteli* ◊*Pseudomonas cuatrocienegasensis* ◊*Pseudomonas protegens* ◊*Pseudomonas stutzeri* ◊*Serratia marcescens* ◊*Shigella flexneri* ◊*Sporosarcina luteola* ◊*Staphylococcus agnetis* ◊*Staphylococcus arlettae* ◊*Staphylococcus caprae* ◊*Staphylococcus epidermidis* ◊*Staphylococcus gallinarum* ◊*Staphylococcus haemolyticus* ◊*Staphylococcus hominis* ◊*Staphylococcus saprophyticus* ◊*Staphylococcus succinus* ◊*Staphylococcus warneri* ◊*Staphylococcus xylosus* ◊*Stenotrophomonas maltophila* ◊*Vagococcus fluvialis*		
	Culex pipiens	**Wolbachia* sp.	**Alicyclobacillus* sp. **Enterobacteriaceae* **Erwinia* sp. **Sphingomonas* sp. ◊*Acidovorax* sp. ◊*Acinetobacter* sp. ◊*Afipia* sp. ◊*Agrobacterium tumefaciens* ◊*Asaia* sp. ◊*Bosea vestrisii* ◊*Carnobacterium maltaromaticum* ◊*Curvibacter* sp.	*Aerobic bacterial cultures MiSeq sequencing of 16S rRNA gene amplicons. Entire organism. Adult females. ◊Culture-independent method and PCR amplification of eubacterial 16S rDNA-Entire organisms. Adults.	*Muturi et al., 2016 ◊Zotzmann et al., 2017

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			◊*Enterococcus* sp. ◊*Escherichia coli Hafnia paralvei* ◊*Kluyvera* sp. ◊*Mesorhizobium* sp. ◊*Methylobacterium* sp. ◊*Pantoaea coffeiphila* ◊*Pseudomonas psychrtolerans* ◊ *Pseudomonas* sp. ◊*Rahnella aquatilis* ◊*Ralstonia insidiosa* ◊*Ralstonia sp.* ◊*Sphingomonas echinoides* ◊*Sphingomonas leidy* ◊*Staphylococcus warneri* ◊◊*Streptococcus thermophilus* ◊*Tatumella ptyseos* ◊*Tatumella* sp.		
	Mansonia africana	◊*Wolbachia* sp.	*Aeromonas* sp. *Acinetobacter* sp. *Zymobacter* sp. ◊*Asaia* sp.	*454 pyrosequencing of 16S rRNA. Gut. Adults females. ◊The gene encoding the surface protein of *Wolbachia*, wsp. Guts. Adults females.	*Osei-Poku et al., 2012a ◊Osei-Poku et al., 2012b
	Mansonia uniformis	◊*Wolbachia* sp. •*Rickettsia felis*	*Aeromonas* sp. *Acinetobacter* sp. *Gluconobacter* sp. ◊*Asaia* sp.	*454 pyrosequencing of 16S rRNA.Gut. Adults females. ◊The gene encoding the surface protein of *Wolbachia*, wsp. Gut. Adults females. •Culture-independent methods. Quantitative real-time reverse transcription PCR (qRT-PCR). Entire organisms. Larvae and adults.	*Osei-Poku et al., 2012a ◊Osei-Poku et al., 2012b •Mediannikov et al., 2013
Diptera: **Nematocera** **Psychodidae** **(sand flies)**	*Phlebotomus papatasi*		*Bacillus badius *Bacillus cereus *Bacillus clausii *Bacillus endophyticus *Bacillus flexus *Bacillus licheniformis *Bacillus pumilus *Bacillus subtilis *Brevibacillus brevis *Brevibacillus reuszeri *Enterobacter aerogenes *Lysinibacillus boronitolerans *Lysinibacillus fusiformis *Oceanibacillus sp. *Paenibacillus sp. *Plantibacter flavus *Solibacillus sp. *Staphylococcus saprophyticus *Terribacilus. saccharophilus ◊Edwardsiella ictaluri ◊Enterobacter amnigenus ◊Enterobacter gergoviae ◊Escherichia blattae ◊Klebsiella oxytoca ◊Klebsiella planticola ◊Klebsiella pneumoniae ◊Kluyvera ascorbata ◊Leminorella grimontii ◊Pantoea agglomerans	*Cultured methods and identified using 16S ribosomal DNA analysis-Guts-Adults females. ◊Biochemical reactions and 16 s rDNA sequencing. Gut and entire organisms. Immature and adults.	*Mukhopadhyay et al., 2012 ◊Maleki-Ravasan et al., 2014

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			◊*Proteus mirabilis* ◊*Proteus vulgaris* ◊*Providencia alcalifaciens* ◊*Providencia rettgeri* ◊*Pseudomonas aeruginosa* ◊*Rahnella aquatilis* ◊*Serratia plymuthica* ◊*Shigella dysenteriae* ◊*Staphylococcus aereus* ◊*Tatumella ptyseos* ◊*Yersenia pseudotubercolosis*		
	Phlebotomus argentipes		*Acinetobacter* sp. *Bacillus cereus* *Bacillus* sp. *Bacillus megaterium* *Bacillus mycoides* *Bacillus niacini* *Bacillus pumilus* *Bacillus subtilis* *Brevibacterium casei* *Brevibacterium iodinum* *Brevibacterium linens* *Cellulomonas* sp. *Enterobacter aerogenes* *Enterobacter cloacae* *Escherichia coli* *Gordonia terrae* *Microbacterium oxydans* *Microbacterium* sp. *Micrococcus tetragenes* *Pseudomonas sp.* *Staphylococcus arlettae* *Staphylococcus aureus* *Staphylococcus citreus* *Staphylococcus cohnii* *Staphylococcus nepalensis* *Staphylococcus* sp. *Stenotrophomonas maltophilia*	Cultured methods and 16S rDNA PCR amplifications. Gut and entire organisms. Adults.	Hillesland et al., 2008
	Phlebotomus duboscqi		*Citrobacter* sp. *Ochrobactrum anthropi* *Serratia marcescens* *Stenotrophomonas maltophilia*	Cultured methods, standard bacteriological methods and biochemical properties of the isolates. Gut. Fourth-instar larvae, pupae and freshly emerged females.	Volf et al., 2002
	Phlebotomus perniciosus	*Wolbachia* sp.	*Bacillus casmanesis* *Bacillus galactosidilyticus* *Bacillus oleronius* *Bacillus* sp. *Bacillus subtilis* *Bordetella avium* *Brevundimonas terrae* *Burkholderia fungotum* *Ehrlichia* sp. *Enterococcus faecalis* *Kocuria polaris* *Lysinibacillus* sp.	Culture-dependent (Amplification of the 16S rDNA gene) and culture-independent (denaturing gradient gel electrophoresis) methods. Adults females.	Fraihi et al., 2017

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			Microbacterium sp. *Micrococcus* sp. *Nocardia ignorata* *Ochrobactrum intermedium* *Ochrobactrum* sp. *Rhizobium pusense* *Roseomonas ludipueritiae* *Saccharomonospora* sp. *Serratia* sp. *Sporosarcina koreensis* *Staphyloccocus epidermidis* *Stenotrophomonas maltophilia* *Veillonella* sp.		
	Phlebotomus chinensis	*Coxiella* sp. *Rickettsiella* sp. *Diplorickettsia* sp. *Spiroplasma* sp. *Wolbachia* sp.	*Cedecea* sp. *Citrobacter* sp. *Enterobacter* sp. *Enterococcus* sp. *Erwinia* sp. *Escherichia* sp. *Klebsiella* sp. *Pantoea* sp. *Pluralibacter* sp. *Pseudomonas* sp. *Sphingomonas* sp.	Culture independent and dependent approaches. Amplify 16S ribosomal RNA gene fragment. Entire organism. Adults.	Li et al., 2016
	Lutzomyia evansi	◊*Wolbachia* sp.	**Acinetobacter calcoaceticus* **Acinetobacter gyllenbergi* **Bacillius thuringiensis* **Bacillus anthracis* **Bacillus gibsonii* **Bacillus* sp. **Bacillus megaterium* **Brevibacterium linens* **Burkholderia cenocepacia* **Enterobacter aerogenes* **Enterobacter cancerogenus* **Enterobacter cloacae* **Enterobacter hormaechei* **Enterobacter ludwigii* **Microbacterium foliorum* **Microbacterium pseudoresistens* **Ochrobactrum anthropi* **Olivibacter soli* **Paenibacillus illinoisensis* **Pantoea ananatis* **Pseudomonas otitidis* **Pseudomonas aeruginosa* **Pseudomonas putida* **Rummeliibacillus stabekisii* **Shinella zoogloeoides* **Staphylococcus agnetis* * *Staphylococcus arlettae* **Staphylococcus epidermidis* * *Staphylococcus sciuri*	*Culture-dependent techniques and culture-independent (denaturing gradient gel electrophoresis) methods. Gut-Larvae, pupae and adults. ◊Cloning and DNA fragment sequencing for *Wolbachia* wsp gene. Torax and abdomen. Adults.	*Vivero et al., 2016 ◊Vivero et al., 2017

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Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			*Streptomyces cinnabarinus * Streptomyces malachitospinus		
	Lutzomyia longipalpis		*Burkholderia cepacia *Citrobacter freundii *Enterobacter aerogenes *Enterobacter amnigenus *Enterobacter cloacae *Enterobacter gergoviae *Enterobacter taylorae *Escherichia coli *Flavimonas aryzihabitans *Klebsiella oxytoca *Morganella morganii *Pantoea agglomerans *Pseudomonas putida *Pseudomonas aeruginosa *Serratia liquefaciens *Serratia marcescens *Stenotrophomonas maltophila *Weeksella virosa ◊Acinetobacter baumannii ◊Acinetobacter sp. ◊Bradyrhizobium japonicum Propionibacterium acnes ◊Caulobacter sp. ◊Clostridium disporicum ◊Dyadobacter ginsengisoli ◊Erwinia billingiae ◊ Klebsiella pneumoniae ◊ Leptothrix sp. ◊Nocardioides albus ◊Pantoea sp. ◊Ralstonia pickettii ◊ Ralstonia sp. ◊Saccharibacter floricola ◊Serratia sp. ◊Sphingobacterium daejeonense	*Molecular and traditional bacteriological methods. Gut and Malpighian tubules. Adults. ◊Denaturing gradient gel electrophoresis of bacterial 16 rRNA gene fragments. Entire organism. Adult females.	Gouveia et al., 2008 ◊Sant'Anna et al., 2012
	Lutzomyia cruzi		*Bradyrhizobium japonicum* *Caulobacter* sp. *Chrysoenbacterium meningosepticum* *Clostridium disporicum* *Clostridium glycolicum* *Lactobacillus zymae* *Ralstonia* sp. *Staphylococcus xylosus*	Denaturing gradient gel electrophoresis of bacterial 16 rRNA gene fragments. Entire organism. Adult females.	Sant'Anna et al., 2012
	Lutzomyia intermedia	*Rickettsia* sp. *Wolbachia* sp.	*Acinetobacter* sp. *Bradyrhizobium* sp. *Curtobacterium* sp. *Enterobacter* sp. *Erwinia* sp. *Kingella* sp. *Kluyvera* sp. *Ochrobactrum* sp.	16S rRNA V1-V3 region and Illumina sequencing. Gut. Adults	Monteiro et al., 2016

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Table 2 (*continued*)

Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			Pelomonas sp. *Propionibacterium* sp. *Pseudomonas* sp. *Ralstonia* sp. *Serratia* sp.		
Diptera: Brachycera Glossinidae (tse-tse Flies)	*Glossina palpalis palpalis*	●*Wigglesworthia glossinidia* □*Sodalis glossinidius* o*Spiroplasma* sp.	**Acinetobacter* sp. **Enterobacter sp.* **Enterococcus sp.* ◊*Chryseobacterium* sp. ◊*Lactococcus* sp. ◊*Providencia* sp. ◊*Sphingobacterium sp* ◊*Staphylococcus* sp.	*Culture independent and dependent approaches. Amplification of the small ribosomal RNA subunit. Midgut. Adults. ◊Culture independent and dependent approaches. Amplification of 16S rDNA and product of the small ribosomal RNA subunit gene. Midgut. Adults. ●Culture independent and amplification for the ITS-2 and the 16S rDNA. Entire organism or bacteriomes. Adults. □Culture independent and PCR amplification. Entire organism. Adults. oIndependent approach-High throughput sequencing of the 16S rRNA gene. Entire organism. Larvae, freshly emerged adults (teneral) and adults.	*Geiger et al., 2009 ◊Geiger et al., 2011 ●Chen et al., 1999 □Farikou et al., 2010 oDoudoumis et al., 2017
	Glossina palpalis gambiensis	**Wigglesworthia glossinidia* ◊*Sodalis glossinidius* □*Wolbachia* sp.	●*Serratia glossinae* ▫*Serratia* sp. ▫*Klebsiella* sp. ▫*Erwinia* sp. ▫*Trabulsiella* sp. ▫*Pantoea* sp.	*Amplification for the ITS-2 and the 16S rDNA. Entire organism or bacteriomes. Adults. ◊Amplification of a specific PCR fragment using primers GPO1 F and GPO1 R and analysis of the 16S rRNA gene. Hemolymph. Adults females. ●Cultured methods and 16S rRNA gene sequences was amplified and fatty acid profiling. Midgut. Adults. □Independent approach-16S rRNA, wsp and MLST gene markers. Entire organism. Adults. ▫Independent approach. High throughput sequencing of the 16S rRNA gene-Entire organisms- Larvae, freshly emerged adults (teneral) and adults.	*Chen et al., 1999 ◊Geiger et al., 2005 ●Geiger et al., 2010 □Doudoumis et al., 2012 ▫Doudoumis et al., 2017
	Glossina nigrofusca	◊*Sodalis glossinidius*	**Enterobacter* sp.	*Culture independent and dependent approaches. Amplification of 16S rDNA and product of the small ribosomal RNA subunit gene. Midgut. Adults. ◊Culture independent and PCR amplification. Entire organism. Adults.	*Geiger et al., 2011 ◊Farikou et al., 2010
	Glossina pallicera	◊*Sodalis glossinidius*	**Acinetobacter* sp. **Enterobacter* sp. **Pseudomonas* sp.	*Culture independent and dependent approaches. Amplification of 16S rDNA and product of the small ribosomal RNA subunit gene. Midgut. ◊Culture independent and PCR amplification-Entire organism. Adults.	*Geiger et al., 2011 ◊Farikou et al., 2010
	Glossina fuscipes fuscipes	**Wigglesworthia glossinidia* ◊*Sodalis glossinidius* ●*Wolbachia* sp. □*Spiroplasma* sp.	**Arthrobacter* sp. **Bacillus endophyticus* **Bacillus* sp. **Bacillus subtilis* **Burkholderia* sp. **Enterobacter* sp. **Exiguobacterium* sp. **Morganella* sp. **Paenibacillus* sp. **Planococcaceae* **Providencia* sp. **Pseudomonas* sp. **Pantoea* sp. **Serratia marcescens* **Staphylococcus epidermidis* * *Staphylococcus* sp.	*Culture independent and dependent approaches. A region of the 16S rRNA gene of the bacterial isolates was amplified. Entire organisms. Adults. ◊Isolation and culture. Sequencing of the 16S rDNA gene. Hemolymph. Adults. ●Independent approach- *Wolbachia*-specific forward primer. Entire organisms-Adults. □Independent approach. High throughput sequencing of the 16S rRNA gene. Entire organism. Larvae, freshly emerged adults (teneral) and adults.	*Lindh and Lehane, 2011 ◊Matthew et al., 2005 ●Alam et al., 2012 □Doudoumis et al., 2017
	Glossina morsitans morsitans	**Wigglesworthia glossinidia* ◊*Wolbachia* sp. ●*Sodalis glossinidius*	◊*Acinetobacter* sp. ◊*Aeromonas* sp. ◊*Brevundimonas* sp. ◊*Flavobacterium* sp. ◊*Pantoea* sp. ◊*Pedobacter* sp. ◊*Propioniobacterium* sp. ◊*Rhodospirillales* ◊*Shewanella* sp. ◊*Streptococcus* sp. ◊*Klebsiella* sp.	*Amplification for the ITS-2 and the 16S rDNA. Entire organism or bacteriomes. Adults. ◊Independent approach. High throughput sequencing of the 16S rRNA gene. Entire organisms. Larvae, freshly emerged adults (teneral) and adults. ●Amplification of a specific PCR fragment using primers GPO1 F and GPO1 R and analysis of the 16S rRNA gene. Hemolymph. Adults females.	*Chen et al., 1999 ◊Doudoumis et al., 2017 ●Geiger et al., 2005

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Table 2 (*continued*)

Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
	Glossina *morsitans* *submortisans*	**Wigglesworthia glossinidia* ◊*Sodalis glossinidius* ◊*Rickettsia* sp.		*Amplification for the ITS-2 and the 16S rDNA. Entire organism or bacteriomes. Adults. ◊Independent approach. High throughput sequencing of the 16S rRNA gene. Entire organism. Larvae, freshly emerged adults (teneral) and adults.	*Chen et al., 1999 ◊Doudoumis et al., 2017
Insecta **Hemiptera** **Triatominae** **(kissing bugs)**	*Rhodnius prolixus*	◊*Arsenophonus* sp.	**Erwinia chrysanthemi* **Serratia marcescens* **Pectobacterium* sp. ◊*Rhodococcus* sp. ◊*Staphylococcus* sp. ◊*Morganella* sp. ◊*Decholoromonas* sp. ◊*Clostridium* sp. •*Rhodococcus rhodnii*	*Cultivation-independent methods. Analysis of 16 s rDNA using denaturing gradient gel electrophoresis and cloned-based sequencing. Guts and feces. 5th instar larvae. ◊Culture independent methods- high throughput sequencing of region V3-V4 of bacterial 16S rRNA gene. Gut and gonad tissue. •Cultured methods. Entire organism, except head and legs. Larvae and adults.	*Da Mota et al., 2012 ◊Díaz et al., 2016 •Ben-Yakir, 1987
	Rhodnius neglectus		*Erwinia chrysanthemi* *Pectobacterium* sp.	Cultivation-independent methods. Analysis of 16 s rDNA using denaturing gradient gel electrophoresis and cloned-based sequencing. Guts and feces. 5th instar larvae.	Da Mota et al., 2012
	Rhodnius pallescens	**Wolbachia* sp.	◊*Bacillales* ◊*Burkholderia* sp. ◊*Enterobacteriaceae* ◊*Gordonia* sp. ◊*Prevotella* sp. ◊*Rhodococcus* sp. ◊*Staphylococcus* sp. ◊*Williamsia* sp.	*Cultivation-independent methods. Specific primers for 16S rRNA and wsp genes. Gonads, salivary glands, and intestines. Nymphs and adults. ◊Cultivation-independent methods. Sequencing of the V4 region of 16S rRNA gene. Guts. Adults.	*Espino et al., 2009 ◊Montoya-Porras et al., 2018
	Rhodnius sp.	*Wolbachia* sp.	*Erwinia chrysanthemi* *Pectobacterium* sp.	Cultivation-independent methods. Analysis of 16 s rDNA using denaturing gradient gel electrophoresis and cloned-based sequencing. Guts and feces. 5th instar larvae.	Da Mota et al., 2012
	Dipetalogaster megistus		*Pectobacterium* sp. *Rohrkolberia cinguli* *Serratia* sp.	Cultivation-independent methods. Analysis of 16 s rDNA using denaturing gradient gel electrophoresis and cloned-based sequencing. Guts and feces. 5th instar larvae.	Da Mota et al., 2012
	Panstrongylus maximus	**Arsenophonus* sp.	◊*Decholoromonas* sp. ◊*Morganella* sp. ◊*Pectobacterium* sp. ◊*Staphylococcus* sp.	*Cultivation-independent methods. Analysis of 16 s rDNA using denaturing gradient gel electrophoresis and cloned-based sequencing. Guts and feces. 5th instar larvae. ◊Culture independent methods. High throughput sequencing of region V3-V4 of bacterial 16S rRNA gene. Gut and gonad tissue.	*Da Mota et al., 2012 ◊Díaz et al., 2016
	Triatoma infestans	**Arsenophonus* sp.	**Serratia* sp. ◊*Clostridium* sp. ◊*Crynebacterium* sp. ◊*Dietzia* sp. ◊*Morganella* sp. ◊*Pectobacterium* sp. ◊*Rhodococcus* sp. ◊*Staphylococcus* sp.	*Cultivation-independent methods. Analysis of 16 s rDNA using denaturing gradient gel electrophoresis and cloned-based sequencing. Guts and feces. 5th instar larvae. ◊Culture independent methods. High throughput sequencing of region V3-V4 of bacterial 16S rRNA gene. Gut and gonad tissue.	*Da Mota et al., 2012 ◊Díaz et al., 2016
	Triatoma vitticeps,	*Arsenophonus* sp.	*Serratia* sp.	Cultivation-independent methods. Analysis of 16 s rDNA using denaturing gradient gel electrophoresis and cloned-based sequencing. Guts and feces. 5th instar larvae.	Da Mota et al., 2012
	Triatoma maculata		*Aeromonas* sp. *Bacteroides* sp. *Burkholderia* sp. *Corynebacterium* sp. *Dietzia* sp. *Kocuria* sp. *Lactococcus* sp. *Nocordia* sp. *Pelomonas* sp. *Planococcaceae* *Prevotella* sp. *Pseudomonas* sp. *Rhodococcus* sp.,	Cultivation-independent methods. Sequencing of the V4 region of 16S rRNA gene. Gut. Adults.	Montoya-Porras et al., 2018

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Table 2 (*continued*)

Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
			Staphylococcus sp. *Veillonella* sp. *Williamsia* sp.		
	Triatoma pseudomaculata		*Acinetobacter* sp. *Actinomyces* sp. *Adhaeribacter* sp. *Chryseobacterium* sp. *Corynebacterium* sp. *Dietzia* sp. *Enterococcus* sp. *Gordonia* sp. *Hydrogeniphilus* sp. *Janthinobacterium* sp. *Marinomonas* sp. *Microvirga* sp. *Mycobacterium* sp. *Propionibacterium* sp. *Pseudomonas* sp. *Rhodococcus* sp. *Serratia* sp. *Shinella* sp. *Sphingomonas* sp. *Stenotrophomonas* sp. *Streptococcus* sp. *Streptphyta* sp. *Xanthobacter* sp. *Williamsia* sp.	Culture independent methods. Amplification of the 16S rRNA, denaturing gradient gel electrophoresis, DNA fragment cloning, Sanger sequencing and 454 pyrosequencing. Gut. Nymphs and adults.	Gumiel et al., 2015
	Triatoma brasiliensis	**Arsenophonus* sp.	**Acinetobacter* sp. **Bradyrhizobium* sp. **Comamonas* sp. **Corynebacterium sp* **Diaphorobacter* sp. **Dietzia* sp. **Geobacillus* sp. **Gordonia* sp. **Haemophilus* sp. **Hydrogeniphilus* sp. **Mycobacterium* sp. **Propionibacterium* sp. **Pseudomonas* sp. **Rhodococcus* sp. **Serratia* sp. **Staphylococcus* sp. **Streptococcus* sp. **Streptphyta* sp. **Williamsia* sp. ◊*Morganella* sp. ◊*Decholoromonas* sp. ◊*Clostridium* sp.	* Culture independent methods. Amplification of the 16S rRNA, denaturing gradient gel electrophoresis, DNA fragment cloning, Sanger sequencing and 454 pyrosequencing. Gut. Nymphs and adults. ◊Culture independent methods. High throughput sequencing of region V3-V4 of bacterial 16S rRNA gene. Gut and gonad tissue.	*Gumiel et al., 2015 ◊Díaz et al., 2016

(*continued on next page*)

Table 2 (*continued*)

Taxa arthropod vectors of human pathogens	Species	Primary symbiont	Secondary symbiont	Methods used to isolation and determination of symbionts, body parts or organs used and stadium of the arthropod vector	References
	Triatoma juazeirensis	*Arsenophonus* sp.	*Clostridium* sp. *Decholoromonas* sp. *Morganella* sp. *Rhodococcus* sp. *Staphylococcus* sp.	Culture independent methods. High throughput sequencing of region V3-V4 of bacterial 16S rRNA gene. Gut and gonad tissue.	Díaz et al., 2016
	Triatoma sherlocki	*Arsenophonus* sp.	*Staphylococcus* sp.	Culture independent methods. High throughput sequencing of region V3-V4 of bacterial 16S rRNA gene. Gut and gonad tissue.	Díaz et al., 2016
Insecta **Hemiptera** **Cimicidae** **(bed bugs)**	*Cimex lectularius*	**Wolbachia* sp. *Coxiella sp. ◊*Midichloria mitochondrii* ●*Rickettsia prowazekii* ●*Rickettsia rickettsii*	**Bacillus* sp. **Enterobacteriaceae* **Methylobacterium* sp. **Pseudomonas* sp. **Shinella* sp. **Sphingomonas* sp. **Staphylococcus* sp. **Streptococcus* sp. □*Burkholderia multivorans* ○*Staphylococcus saprophyticus* ○*Arthrobacter* sp. ○*Micrococcus luteus* ▫*Kuria kristinae* ▫*Micrococcus* sp. ▫*Staphylococcus arlettae* ▫*Staphylococcus epidermidis* ●*Brucella malitensis* ●*Mycobacterium leprae* ●*Mycobacterium tuberculosis* ●*Salmonella typhy*	*Culture-independent amplifying the V4–V6 hypervariable region of the 16S rDNA gene, followed by 454 Titanium sequencing. Entire organism. Adults. □Culture-independent amplifying 16S–23S rRNA intergenic transcribed spacer region-Entire organisms. Adults ○Culture-dependent techniques. 16S rRNA and D2 LSU rDNA genes were sequenced. Abdomen, male intromittent organ and proboscis. Adults Males. ◊Culture-independent-Screening of the Citrate synthase, OmpA genes, ITS2 gene and rrS gene. ▫Culture-dependent techniques and amplification of the 16 s ribosomal RNA (rRNA) gene. Entire organism and gut. Adults. ●Cited in Delaunay et al., 2011 according a review previous of Burton, 1963.	*Meriweather et al., 2013 □Saenz et al., 2013 ○Reinhardt et al., 2005. ◊Richard et al., 2009 ▫Cockburn et al., 2013 ●Delaunay et al., 2011
Insecta **Phthiraptera** **Pediculidae** **(lices)**	*Pediculus h. humanus*	◊*Rickettsia prowazekii* ●*Wolbachia* sp. □*Riesia pediculicola*	◊*Acinetobacter baumannii* **Bartonella quintana* **Serratia marcescens* ▫*Borrelia recurrentis*	*Culture-dependent and independent techniques. Biochemical, microscopic and molecular identification. Half of body. ◊Experimental infections ▫Experimental infections ●Culture independent methods-Amplification of the gene primer wsp-Entire organisms. Adults □Molecular phylogenetic analysis of 16S rRNA gene sequences and In situ hybridization. Adult females.	*La Scola et al., 2001 ◊Houhamdi et al., 2002 ▫Houhamdi and Raoult, 2005 ●Kyei-Poku et al., 2005 □Sasaki-Fukatsu et al., 2006
	Pediculus h. capitis	◊*Wolbachia* sp.	**Bartonella quintana* ●*Acinetobacter baumannii*	*Culture independent methods-and specific primers for *Bartonella*. Entire organism. Adults. ●Culture independent methods -Specific quantitative real-time PCR targeting a fragment of the rpoB gene. Entire organism. Adults. ◊Culture independent methods. Amplification of the gene primer wsp. Entire organism. Adults.	*Sasaki et al., 2006 ●Bouvresse et al., 2011 ◊Kyei-Poku et al., 2005

3. Methods

Literature has been surveyed using Scopus and Web of Science databases (accessed August 2017). As keywords, we used various combinations of "microbiota", "microbiome" and "symbiotic bacteria" with "human blood-feeding arthropods", "arthropods vectors" "insect vectors" "vector-borne diseases" "ticks", "Culicidae", "mosquitoes", "sand flies", "Phlebotominae", "tse-tse flies" " Glossinidae", "Triatominae" "kissing bugs", "*Cimex*", "bed bugs", "*Pediculus humanus*", and "lice". Although our review is rather wide in its literature coverage, we mostly focused on studies that include human blood-feeding arthropods species from field or colonies in laboratory, without consider studies with experimental infections. We considered as human blood-feeding arthropods according to Bogitsh et al. (2013), Garcia (2007) and Vayssier-Taussat et al. (2015). Literature on the topic was double-checked running the same researches on other databases (e.g., Google Scholar) using the keywords detailed above.

4. General patterns of bacterial microbiota in human blood-feeding arthropods

As showed by the literature analyzed here, Diptera (Culicidae, Glossinidae, and Phlebotominae) harbor the highest number of species and genera of symbiont bacteria, while ticks are the second taxa of HBFA with a high number of symbiont bacteria (Table 2). This could be explained considering that Diptera and ticks are indeed broad taxonomic levels including numerous species. On the other hand, other groups of human blood feeding Hemiptera like bedbugs (*C. lectularius* and *C. hemipterus*), body lice (*P. humanus humanus*), head lice (*P. humanus capitis*), and pubic lice (*P. pubis*) only include few species. However, studies on microbiota in mosquitoes and tick species, demonstrate that the number of bacterial species that they may lodge every host species is larger than that in bedbugs, human lice, and triatomines (Bonnet et al., 2017; Minard et al., 2013; van Tol and Dimopoulos, 2016) (Table 2).

Specifically, different studies on HBFA bacterial microbiota and microbiome indicate that mosquitoes are the group among Diptera with more bacterial taxa associated (Ahantarig et al., 2013; Minard et al., 2013; Narasimhan and Fikrig, 2015; Van Tol and Dimopoulos, 2016), even with symbiont bacteria number comparable to ticks (Table 2). Sand flies (genera *Phlebotomus* and *Lutzomyia*) and tse-tse flies (genus *Glossina* spp.) are the next arthropod vector groups harboring the highest number of symbiont bacteria, respectively (Fraihi et al., 2017; Geiger et al., 2013; Geiger et al., 2014; Monteiro et al.,2016; Wang et al., 2013). Whereas the number of bacterial species reported as associated to triatomine hemipterans vectoring *T. cruzi* has increased in recent years, such number remains lower than those reported in other HBFA groups (Azambuja et al., 2005; Da Mota et al., 2012; Díaz et al., 2016; Gumiel et al., 2015; Vallejo et al., 2009). On the other hand, bedbugs, as well as body and head lice, are the groups with the least number of bacterial communities reported (Benoit et al., 2016; Boyd and Reed, 2012; Meriweather et al.,2013; Veracx and Raoult, 2012).

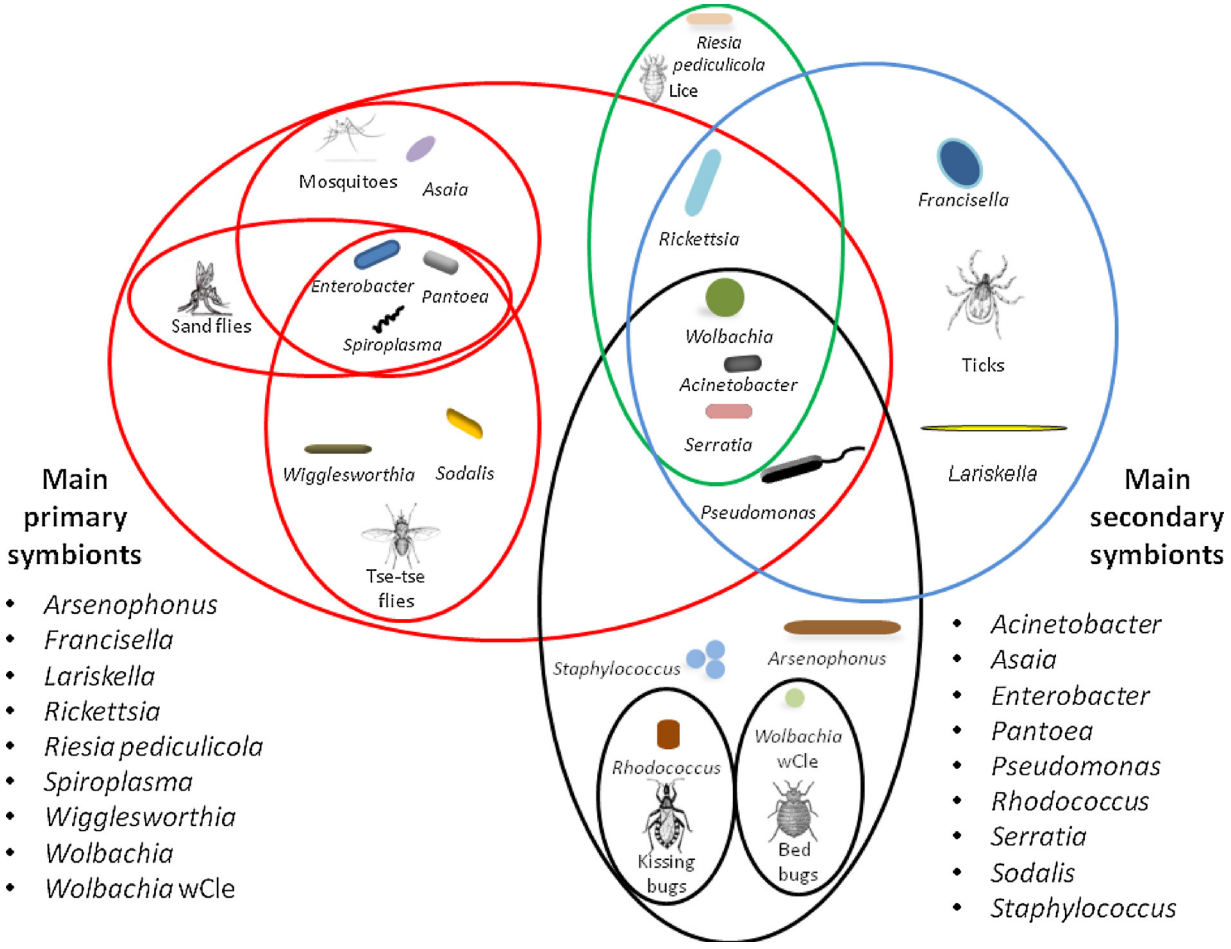

Fig. 1. Overall view of the main groups of primary and secondary symbionts of human blood feeding arthropods. Circles with the same colour are used to relate vectors with their corresponding symbionts. Blue circle Ixodida (ticks), red circle Diptera (mosquitoes, sand flies and tse-tse flies), black circle Hemiptera (kissing bugs and bed bugs) and green circle Phthiraptera (lice). The symbiont bacteria more widespread in all taxa are ubicated in the middle of the diagram: *Wolbachia*, *Acinetobacter* and *Serratia*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

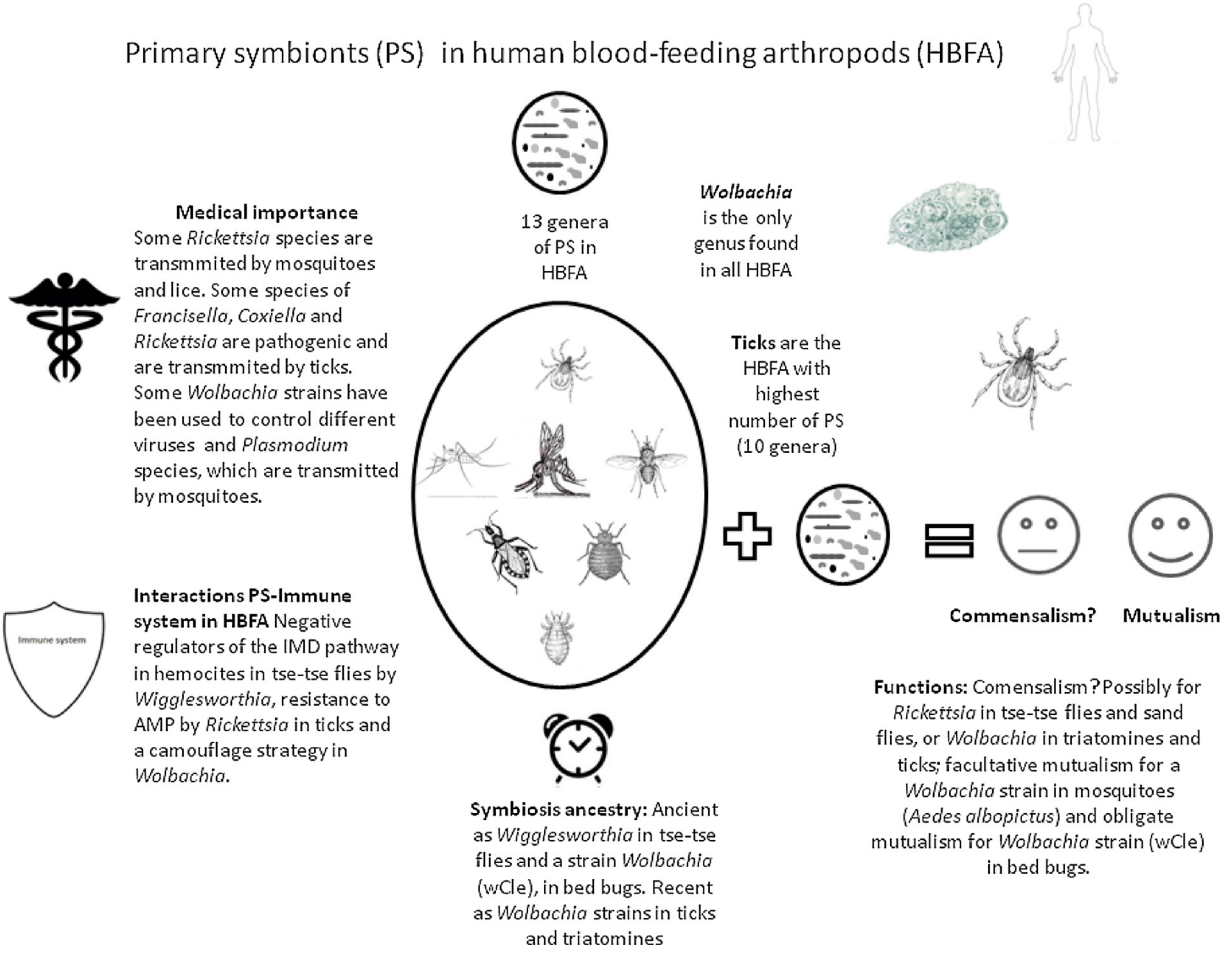
Primary symbionts (PS) in human blood-feeding arthropods (HBFA)
13 genera of PS in HBFA
Wolbachia is the only genus found in all HBFA
Medical importance
Some Rickettsia species are transmmited by mosquitoes and lice. Some species of Francisella, Coxiella and Rickettsia are pathogenic and are transmmited by ticks. Some Wolbachia strains have been used to control different viruses and Plasmodium species, which are transmitted by mosquitoes.
Ticks are the HBFA with highest number of PS (10 genera)
Immune system
Interactions PS-Immune system in HBFA Negative regulators of the IMD pathway in hemocites in tse-tse flies by Wigglesworthia, resistance to AMP by Rickettsia in ticks and a camouflage strategy in Wolbachia.
Commensalism?
Mutualism
Functions: Comensalism? Possibly for Rickettsia in tse-tse flies and sand flies, or Wolbachia in triatomines and ticks; facultative mutualism for a Wolbachia strain in mosquitoes (Aedes albopictus) and obligate mutualism for Wolbachia strain (wCle) in bed bugs.
Symbiosis ancestry: Ancient as Wigglesworthia in tse-tse flies and a strain Wolbachia (wCle), in bed bugs. Recent as Wolbachia strains in ticks and triatomines

Fig. 2. Total number of genera of PS registered in HBFA, genus of PS that infect all taxa of HBFA and taxon of HBFA with highest number of PS. Main functions of PS, interactions with immune system arthropod and medical importance are summarized.

Despite the differences in the number of bacterial taxa recorded in HBFA, the dominant bacterial taxon in all HBFA groups is the phylum Proteobacteria. Nevertheless, this does not seem to be a distinctive trait of this feeding guild (i.e., blood ingestion), since this bacterial phylum is predominant in the whole class Insecta, and – in general – in terrestrial arthropods (Douglas, 2015; Vavre and Braig, 2012). To date, no single reason can explain the fact that Proteobacteria is the predominant bacterial phylum in terrestrial arthropods. It has been hypothesized that the wide distribution of γ-Proteobacteria in all terrestrial ecosystems, the high rate of horizontal gene transference, and the peculiar immune system of arthropods have contributed to establish such pattern (Vavre and Braig, 2012).

Although some years ago the genus *Rhodococcus* (phylum Actinobacteria: Actinomycetales) had been reported as predominant in triatomine species *Panstrongylus megistus* Burmeister, *Rhodnius prolixus* Stål, and *Triatoma infestans* Klug (Vallejo et al., 2009), recent studies found that the phylum Proteobacteria is also the main taxon in the triatomine species as *Rhodnius neglectus*, *R. prolixus, Triatoma vitticeps, T. infestans, T. brasiliensis*, *T. pseudomaculata*, *Dipetalogaster maximus*, and *Panstrongylus megistus* (Table 2) (Da Mota et al., 2012; Díaz et al., 2016; Gumiel et al., 2015). The phylum Firmicutes is the second top bacterial group in ticks, Diptera (mosquitoes, sand flies and tse-tse flies) and Hemiptera (triatomines). Although, in mosquitoes the number of genera of Firmicutes and Actinobacteria is the same (Table 2). The third top bacterial nucleus is the phylum Actinobacteria in ticks, Diptera (mosquitoes, flies sand and tse-tse flies), and Hemiptera (triatomines). In general, there are few differences in the number of genera between Firmicutes and Actinobacteria, generating interchange of positions between the second and the third top bacteria nucleus, as was reported previously in some investigations (Ahantarig et al., 2013; da Mota et al., 2012; Díaz et al., 2016; Duron et al., 2017; Fraihi et al., 2017; Geiger et al., 2013; Gumiel et al., 2015; Minard et al., 2013; Monteiro et al., 2016; Narasimhan and Fikrig, 2015; Van Tol and Dimopoulos, 2016). In the case of lice, over 90% of bacterial species reported to date belong to the phylum Proteobacteria (Meriweather et al., 2013; Veracx and Raoult, 2012).

5. Primary symbionts shared by HBFA: functions and general patterns by arthropod taxon

Wolbachia is the only bacterial genus that has been reported in all taxa of hematophagous arthropods analyzed here (Figs. 1 and 2). Again, this is not exclusive to the hematophagous feeding guild in arthropods (Benelli et al., 2016b), since *Wolbachia* is found in about 70% of the insect species studied, several crustacean groups (mainly terrestrial ones), chelicerates, and nematodes (Zug and Hammerstein, 2015). Although it has been reported to lodge in various tissues and organs, like salivary glands and gut in ticks and triatomines (Da Mota et al., 2012; Espino et al., 2009; Narasimhan and Fikrig, 2015) and even in *Aedes albopictus* Skuse hemolymph (Dobson et al., 1999), the organs most frequently reported as infected by *Wolbachia* are ovaries and testicles, as well as germline tissues (Zug and Hammerstein, 2015).

Until a few years ago, *Wolbachia* was classified as a reproductive parasite, causing alterations like cytoplasmic incompatibility in the infected invertebrate hosts (Brownlie and Jonhson, 2009; Zug and Hammerstein, 2015). For instance, a higher mortality has been

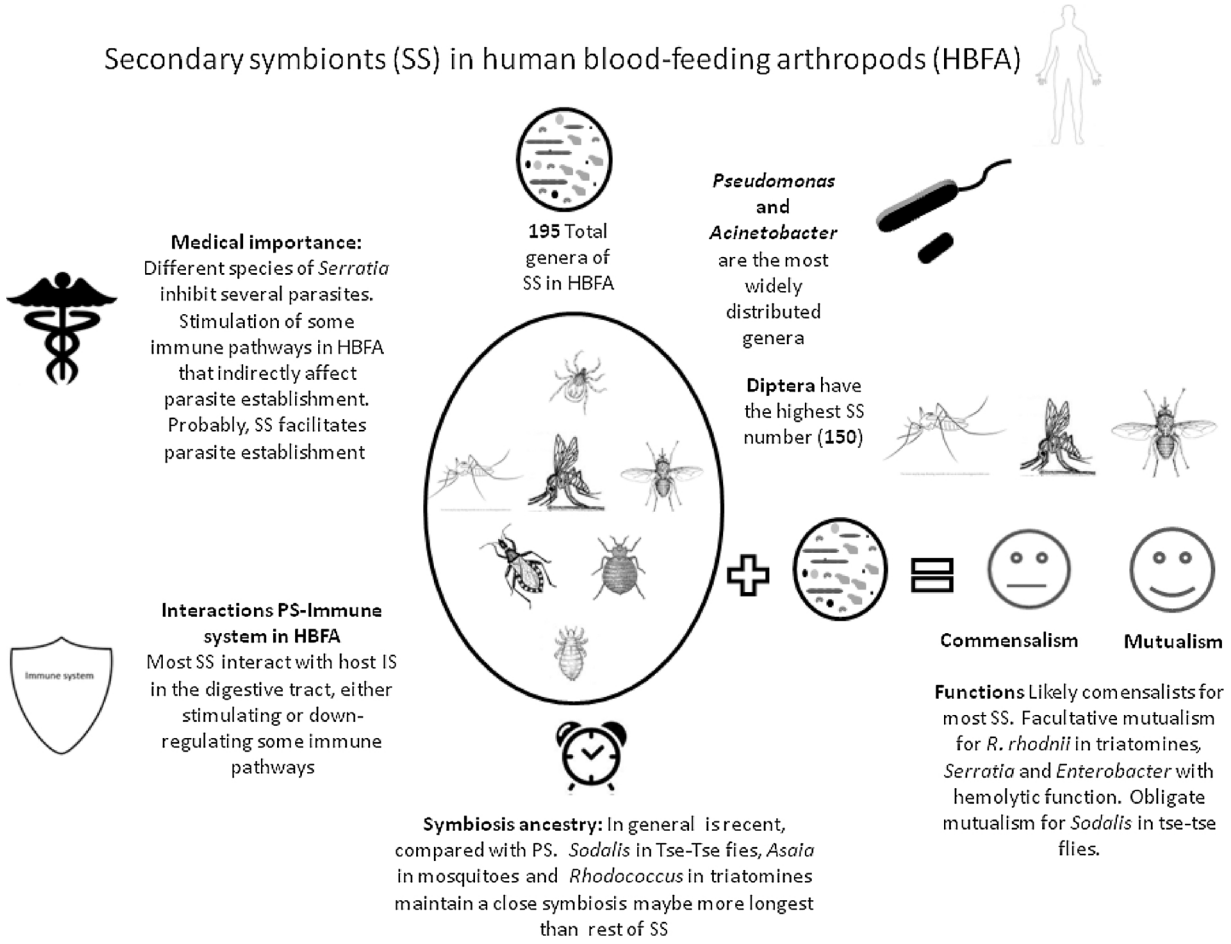
Secondary symbionts (SS) in human blood-feeding arthropods (HBFA)
195 Total genera of SS in HBFA
Pseudomonas and Acinetobacter are the most widely distributed genera
Medical importance: Different species of Serratia inhibit several parasites. Stimulation of some immune pathways in HBFA that indirectly affect parasite establishment. Probably, SS facilitates parasite establishment
Diptera have the highest SS number (150)
Interactions PS-Immune system in HBFA Most SS interact with host IS in the digestive tract, either stimulating or down-regulating some immune pathways
Immune system
Commensalism
Mutualism
Functions Likely comensalists for most SS. Facultative mutualism for R. rhodnii in triatomines, Serratia and Enterobacter with hemolytic function. Obligate mutualism for Sodalis in tse-tse flies.
Symbiosis ancestry: In general is recent, compared with PS. Sodalis in Tse-Tse fies, Asaia in mosquitoes and Rhodococcus in triatomines maintain a close symbiosis maybe more longest than rest of SS

Fig. 3. Total number of genera of SS registered in HBFA, genera of SS that infect all taxa of HBFA and taxon of HBFA with highest number of SS. Main functions of SS, interactions with immune system arthropod and medical importance are summarized.

observed in tse-tse fly embryos (*Glossina morsitans* Westwood) when *Wolbachia*-infected males and females mate with non-infected females and males, respectively (Alam et al., 2011). On the other hand, a facultative mutualism of several *Wolbachia* strains has been reported in *Ae. albopictus* mosquitoes, where infected populations exhibit increased survival, longevity, and/or fertility (Dobson et al., 2004). Interestingly, two *Wolbachia* strains with different evolutionary traits have been detected in human lice; while the strains circulating in America and Europe correspond to the groups A and B, Australian *Wolbachia* strains are in the group F, a strain usually related to infection in nematodes (Perotti et al., 2009).

The evolution of the genus *Wolbachia* has resulted in the strain wCle, which acts as an obligate mutualist when infecting *C. lectularius* bedbugs (Hosokawa et al., 2010). In contrast with most *Wolbachia* strains, which have been described as strict parasites in arthropods, wCle provides biotin and riboflavin and is involved in reproductive aspects of its host, because its removal is accompanied by higher offspring mortality rates and sterility (Hosokawa et al., 2010; Nikoh et al., 2014). wCle is harbored in bacteriocytes adjacent to male and female gonads, and thus the main transmission mode is vertical (Hosokawa et al., 2010). Notably, *Wolbachia* may not be regarded as a true symbiont in ticks, since several studies suggest that the infection would be explained by hymenopteran parasitoids (Bonnet et al., 2017), as it has been documented in the castor bean tick, *Ixodes ricinus* Linnaeus, host to the hymenopter *Ixodiphagus hookeri* Howard (Plantard et al., 2012). Only recently *Wolbachia* has been detected in some species of sand flies and triatomines (Da Mota et al., 2012; Fraihi et al.,2017; Vivero et al., 2017), and much of its function in both insect groups remains unknown.

The genus *Rickettsia* (α-Proteobacteria: Rickettsiales) is the second most widely distributed bacterial group in the whole phylum Arthropoda (Gottlieb et al., 2012). It has infected and coevolved with some of the HBFA groups discussed in this work, including the body lice *P. humanus humanus*, the tse-tse fly *Glossina morsitans submorsitans* Newstead, *Ae. albopictus*, *Anopheles gambiae* Giles, *Anopheles funestus* Giles and *Mansonia africana* Theobald mosquitoes, several tick species and the sand fly *Phlebotomus chinensis* Newstead (Dieme et al., 2015; Duron et al., 2017; Fraihi et al., 2017; Li et al., 2016; Socolovschi et al., 2012a,b; Mediannikov et al., 2013; Narasimhan and Fikrig, 2015; Veracx and Raoult, 2012). Additionally, the presence of *Rickettsia* has been reported in species of hematophagous arthropods that do not feed exclusively from humans, like fleas and mites (i.e., *Ornithonyssus bacoti* Hirst, *Liponyssoides sanguineus* Levine and Lage, and *Neotrombicula* sp. Hirst) (Jofré et al., 2009).

Rickettsia has widely co-diversified in tick hosts, with a wide variety of symbiotic relationships, ranging from pathogenicity to mutualism. A few cases more than one *Rickettsia* strain has been found in some tick species (Table 2). Previous investigations reported that when *Rickettsia* infects an individual or a population of hosts, usually there is no co-infection by *Coxiella* (γ-Proteobacteria: Legionellales), an endosymbiont that has closely co-evolved with ticks as well (Duron et al., 2017). However, we found both bacteria genera in several tick species that feed on humans (Fig. 1, Table 2). The evolution of this genus has been variable in all other HBFA taxa. In the body lice *P. humanus humanus*, *Rickettsia prowazekii* has underwent a significant genome reduction. Some researchers have suggested that this could have an impact on the

increased pathogenicity of infected insects towards humans (Veracx and Raoult, 2012). It was recently described that *Rickettsia felis*, the etiologic agent of the flea-borne spotted fever, can naturally infect mosquitoes (*An. gambiae*), while a new *Rickettsia* species closely related to *R. felis* and *Rickettsia hoogstraalii* has been described in tse-tse flies, *G. morsitans morsitans* Wiedemann (Dieme et al., 2015; Mediannikov et al., 2013). It has been experimentally demonstrated that *R. felis* infects and persists in various organs and even in feces of *An. gambiae*, infecting laboratory mice, and thus the latter could be a potential vector for *R. felis* under natural conditions (Dieme et al., 2015). Finally, the presence of a *Rickettsia* strain in a population of the sand fly, *Phlebotomus chinensis* Newstead, was recently reported in China (Li et al., 2016).

Recent genomic analyses on various arthropod species and their associated microbiota, highlighted that *Arsenophonus* (γ-Proteobacteria: Enterobacteriales) is one of the genera of endosymbiont bacteria most widely distributed in arthropods (Duron et al., 2017; Wilkes et al., 2012). In HBFA, *Arsenophonus* sp. has been reported in four tick species and seven triatomine species, infecting several organs and tissues both intra- and extracellularly (Hypša and Dale, 1997; Šorfová et al., 2008; see Table 2). No clear function has been determined to date for *Arsenophonus triatominarum* in triatomines, since non-infected specimens failed to show negative effects on fitness (Wilkes et al., 2012). Not much is known about the function of the genus *Arsenophonus* in ticks, but its presence could be a result from parasitoid infection, as it is the case with *Wolbachia* (Bonnet et al., 2017). Evidence of vertical transference of *Arsenophonus* genes was recently found in the genome of bedbug *C. lectularius* (Benoit et al., 2016).

Even though *Midichloria mitochondrii* (α-Proteobacteria: Rickettsiales) was originally described in the cytoplasm of ovarian cells from the castor bean tick *I. ricinus*, later studies demonstrated its presence in other two tick species feeding on humans, in *C. lectularius*, and in some species of hematophagous arthropods that do not feed exclusively on humans (Beninati et al., 2004; Duron et al., 2017; Pistone et al., 2012; Richard et al., 2009). Concerning the possible role of *M. mitochondrii* in its hosts, most available information is from studies performed on *I. ricinus* ticks, suggesting positive functions like the synthesis of metabolites required under various stress conditions, a role in molting, or conferring resistance against pathogens (Sassera et al., 2006; Pistone et al., 2012).

Various strains of the genus *Coxiella* (γ-Proteobacteria: Legionellales) seem to have coevolved with ticks, even though there are few examples where the effects on fitness of its removal from the host have been analyzed. One of such examples is the lone star tick *Amblyomma americanum* Linnaeus. When *Coxiella* sp. was removed from female ticks, a decrease in successful larval hatching and in the viability of survivors was observed (Zhong et al., 2007). A *Coxiella* strain was recently reported in *C. lectularius* bedbugs and *P. chinensis* sand flies, thus its distribution could be wider than previously thought (Benoit et al., 2016; Li et al., 2016).

Global analyses on each HBFA group considered in this review (Acari, Diptera, Hemiptera, and Phthiraptera) show that ticks are the reservoir of the greatest number of bacterial PS genera: *Rickettsia*, *Coxiella, Francisella*, *Midichloria, Cardinium, Spiroplasma, Arsenophonus*, *Wolbachia, Rickettsiella*, and *Lariskella* (Bonnet et al., 2017) (Fig. 2, Table 2). This diversity could be attributed to the time elapsed since the origin of ticks, preceding even hemipterans, the oldest insects, by about 300 million years (Gaunt and Miles, 2002; Sonenshine and Roe, 2014). In theory, such a lengthy interaction provided a greater opportunity for more endosymbiotic mutualist bacteria to evolve. A prediction of this hypothesis is that most endosymbiont bacteria in ticks would be exclusive of the group and would be absent from other groups of HBFA or hematophagous arthropods feeding from other vertebrates (Vavre and Braig, 2012). However, this is not the case, since except for the genus *Francisella* (γ-Proteobacteria) and very probably *Lariskella* (α-Proteobacteria), all other species of endosymbiont bacteria have been reported in at least another hematophagous arthropod and/or HBFA group (Fig. 1, Table 2). Recently, five of these genera were determined as very probably being PS, although *Coxiella* is the only genus that has maintained a stable symbiosis with ticks for millions of years, successfully diversifying within the group (Duron et al., 2017). Interestingly, several events of PS substitution seem to have occurred in ticks, being *Coxiella* replaced by other four endosymbiont species. These four species could be performing the same function that *Coxiella* had (mainly, B vitamin supplementation in ticks) (Duron et al., 2017). Such pattern, characteristic of this arthropod group, could be a result of the fact that several tick species occasionally feed from other vertebrates, not exclusively from human blood.

While it may seem reasonable that all hematophagous dipterans feeding from humans (Culicidae, Glossinidae and Phlebotominae) would harbor several PS species given their common evolutionary origin and life cycle and their membership to the same trophic guild, each genus exhibits peculiar traits. First, no bacteriocytes where PS could be lodged have been identified in mosquitoes (Minard et al., 2013). Despite this, the genera *Wolbachia*, *Spiroplasma,* and *Rickettsia* have been found in mosquitoes, as well as in tse-tse flies and sand flies (Doudoumis et al., 2017; Fraihi et al., 2017; Minard et al., 2013) (Fig. 1, Table 2). In mosquitoes, *Wolbachia* naturally infects *Ae. albopictus*, *Culex pipiens* Linnaeus*, Culex quinquefasciatus* Say*, Mansonia uniformis* Theobald and *M. africana* although *Ae. aegypti* though it has been demonstrated that *Wolbachia* is not harbored in wild populations (Van Tol and Dimopoulos, 2016; see Table 2).

A wide range of functions have been found for *Wolbachia* in mosquitoes, including modulation of host reproduction, protection against parasite invasion, and resistance to insecticides (Minard et al., 2013). In sand flies, it was recently concluded that *Wolbachia* infects the genera *Phlebotomus* and *Lutzomyia* under natural conditions, but its function and interaction with the host and with other microbial species are still unknown (Fraihi et al., 2017; Matsumoto et al., 2008; Monteiro et al., 2016; Vivero et al., 2016). *Spiroplasma* was known to infect mosquitoes (genus *Anopheles*) and *P. chinensis* sand flies, but it was recently reported to infect several species of tse-tse flies (*Glossina fuscipes fuscipes* and *Glossina tachinoides*), both in wild populations and in the laboratory (Doudoumis et al., 2017; Fraihi et al., 2017; Minard et al., 2013). *Spiroplasma* has a wide range of effects in its arthropod hosts, from protection against the invasion of nematode parasites in the fly *Drosophila neostestacea* Grimaldi, James and Jaenike, 1992 to pathogenicity in *Ae. aegypti* (Humphery-Smith et al., 1991; Jaenike et al., 2010).

Wolbachia and *Riesia pediculicola* (γ-Proteobacteria: Enterobacteriaceae) are the two endosymbiont genera most frequently found in *P. humanus humanus* (Fig. 1, Table 2). However, other reports focused on the presence of the endosymbiont *Rickettsia prowazekii*, the causative agent of epidemic typhus, as well as *Borrelia recurrentis* (Spirochaetes) and *Bartonella quintana* (α-Proteobacteria), causative agents of relapsing fever and trench fever in humans, respectively (Badiaga and Brouqui, 2012).

Finally, *Wolbachia* is the only PS genus shared by hematophagous hemipterans feeding from humans (bedbugs and triatomines), even though the evolution of this bacterial genus in each clade has been very particular. While *Wolbachia* is an obliged mutualist in bedbugs, its function in triatomines has not been elucidated (Fig. 1, Table 2) (Da Mota et al., 2012; Hosokawa et al., 2010).

6. Primary and secondary symbionts exclusive of each group of HBFA

The best-known example, where most criteria to consider a species as a PS in HBFA are met, is *Wigglesworthia glossidinia* (γ-Proteobacteria: Enterobacteriales) (Fig. 1). This genus infects most of tse-tse flies species (*Glossina* spp.) vectoring *Trypanosoma brucei*, the etiologic agent for sleep disease in Africa (Wang et al., 2013; Weiss et al., 2012). Phylogenetic analyses indicate that the symbiosis *Wigglesworthia-Glossina* is at least 50 million-years-old (Chen et al., 1999). Two main *Wigglesworthia*

populations have been identified: one is located in bacteriomes at the anterior midgut, and the other is in extracellular locations at the lumen of the milk gland (the organ responsible for feeding the offspring within the mother) in females, which makes the symbiont transmission vertical (Wang et al., 2013; Weiss et al., 2012). *Wigglesworthia glossidinia* is known to have nutritive, reproductive, and immune functions in tse-tse flies, as well as contributing to develop the peritrophic matrix in the digestive tract (Geiger et al., 2014; Rio et al., 2016; Wang et al., 2009; Weiss et al., 2012).

Sodalis glossinidius (γ-Proteobacteria: Enterobacteriales) is another bacterium frequently found in several species of tse-tse flies (Geiger et al., 2014; Wang et al., 2013; Weiss et al.,2012) (Figs. 1 and 3). *Sodalis glossinidius* has been classified as a SS, since it failed to show a high affinity for any specific organ in the fly, distributing instead in several organs like gut, salivary glands, hemolymph, and muscle, with variable prevalence under natural conditions (Geiger et al., 2014; Weiss et al., 2012). While the genus *Sodalis* has not been reported in other species of hematophagous arthropods feeding exclusively from humans, it has been described in other species of hematophagous insects feeding on mammals and birds, like pigeon lice, flies of the family Hippoboscidae and recently, aquatic mammal lice (Chrudimský et al., 2012; Fukatsu et al., 2007; Nováková and Hypša, 2007).

No definitive evidence indicates a clear function of *Sodalis* in tse-tse flies, but some data suggest that when it is removed from its hosts, their longevity and/or fecundity is affected, and it may result in an increased susceptibility to infection by *T. brucei* under natural conditions (Dale and Welburn, 2001; Farikou et al., 2010).

A new, free-living *Sodalis* species has been recently described (Chari et al., 2015). This species was compared with other *Sodalis* species acting as SS in hematophagous insects, demonstrating the loss of genes in symbiont *Sodalis* species, which show a close interaction with the host (Chari et al., 2015; Boyd et al., 2016). However, the genes that control the pathway for the synthesis of pantothenate (vitamin B5), required for the synthesis of B vitamins, are conserved which is a common trait in many species of symbiont bacteria (Boyd et al., 2016; Chari et al., 2015;).

Male head lice (*Pediculus humanus capitis*) and body lice (*Pediculus humanus humanus*) have a bacteriome (stomach disks) in the midgut, where bacteriocytes lodging *Riesia pediculicola* are located (Kirkness et al., 2010; Veracx and Raoult, 2012) (Fig. 1). In female lice, *R. pediculicola* is located in the lateral oviducts and in the rear part of oocyte poles, being maternal transfer its main mode of transmission (Kirkness et al., 2010). With respect to its function in the host, it is related to the production of pantothenic acid in both sexes, while positive effects of infection on offspring survival and the production of healthy eggs have been reported in females (Kirkness et al., 2010; Rio et al., 2016). Genomic analysis revealed a significant reduction in the size of *R. pediculicola* genome, with less than 600 genes in a short, linear chromosome and a circular plasmid (Kirkness et al., 2010). Among the genes conserved in *R. pediculicola* are those involved in the synthesis of pantothenic acid, while lost genes include those involved in the production of exonucleases and enzymes involved in energy-producing metabolism, which confirms its close relationship with the host (Kirkness et al., 2010).

Ticks harbor a genus of endosymbiont bacteria that has not been found in any other group of hematophagous feeding arthropods. It is the genus *Francisella* (γ-Proteobacteria: Thiotrichales) (Fig. 1, Table 2). While *Francisella* was initially thought to have a much more restricted distribution in ticks, being found mainly in the genus *Dermacentor* Koch (Ahantarig et al., 2013), a recent study found that *Francisella* is infecting other tick genera as well, like *Ixodes* and several species of the genus *Amblyomma* Koch, along with other tick species that do not feed exclusively on humans (Duron et al., 2017). Earlier studies showed that this endosymbiont was closely related with *Francisella tularensis*, the etiologic agent of the zoonotic disease tularemia. Based on this, it has been suggested that *Francisella* could have been acquired as a human pathogen (Duron et al., 2017). The genome of *Francisella* species that is endosymbiont in *Amblyomma maculatum* Koch has been recently sequenced, and virulence-related pseudogenes present in species pathogenic to mammals, like *F. tularensis* and *F. novicida*, were found. This proves the initial hypothesis that the above-mentioned symbiosis had its origin in a pathogen (Gerhart et al., 2016). Researchers also proposed that the *Francisella* species infecting *D. maculatum* has been an endosymbiont only for a short time, and it probably replaced another endosymbiont species like *Coxiella* or *Rickettsia*, widely distributed in ticks (Fig. 1) (Duron et al., 2017).

Rhodococcus rhodnii (Actinobacteria: Nocardiaceae) has been recurrently found exclusively in several triatomine species from South America (Vallejo et al., 2009) (Figs. 1, 3). However, some studies performed with non-culture methods have concluded it is absent in triatomine species where its presence had been previously reported (Da Mota et al., 2012). Such variable prevalence in natural triatomine populations, its extracellular location in the host gut, and a horizontal transmission via coprophagy or cannibalism, indicate that it is very probably a SS species. Sequences coding for the biosynthesis of other B vitamins have been recently found in the genome of *R. rhodnii* (Pachebat et al., 2013). However, being a SS, its absence could be replaced by other species.

Asaia (α-Proteobacteria: Actinobacteria) seemed to be the only bacterial genus that could be regarded as characteristic of mosquitoes (Ahantarig et al., 2013; Van Tol and Dimopoulos, 2016) (Figs. 1, 3). *Asaia* is located in the gut, male and female gonads, and salivary glands of the host. As for *R. rhodnii* in triatomines, *Asaia* transmission is horizontal and its prevalence is variable in the species of the genera *Aedes*, *Anopheles*, *Culex,* and *Mansonia*, indicating that it is also a SS species (Minard et al., 2013; Van Tol and Dimopoulos, 2016).

7. Molecular mechanisms underlying the symbiosis between bacteria and HBFA

There are several open questions about the initial moments of the arthropod-bacteria interaction and on how to differentiate a pathogen species from a non-pathogen one. However, it is very likely that most species currently found as PS or SS were acquired from the environment, and the arthropod IS has played a key role in this process. The IS in arthropods is innate, it is constituted by a cellular response (hemocytes) and a humoral component (Park and Lee, 2011). Once the non-immune barriers (exoskeleton) were overcome, each pathogen, parasite, or parasitoid exhibits a particular marker of pathogen-associated molecular pattern (PAMP), which is recognized by pattern recognition receptors (PRRs) in the arthropod host (Park and Lee, 2011; Baxter et al., 2017). When PAMPs are detected, PRRs activate effector proteins in hemolymph or in cell cytoplasm, and transduction signals are generated in various tissues. Among the main signaling pathways we know the immunodeficient (IMD) pathway, which is activated by Gram-positive bacteria and fungi, and the Toll pathway, activated by Gram-negative bacteria. Once activated, both pathways lead to the production of antimicrobial peptides (AMPs) (Park and Lee, 2011). The Janus Kinase/Signal transducer and activator of transcription (JAK/STAT), another important pathway in the arthropod immune response, is activated mainly by viruses and parasitoid infections (Park and Lee, 2011). With respect to the cellular response, hemocytes are responsible for phagocyting, encapsulating, and nodulating (i.e., gathering several hemocyte layers) foreign agents ranging from small particles to multicellular parasites like nematodes or parasitoid eggs (Strand, 2008). Another important immune response, found only in insects but not in chelicerates (ticks), is the phenoloxidase (PO) cascade (Anjugam et al., 2017), which results in the melanization of the foreign agent and the generation of toxic intermediary products like reactive oxygen species (ROS) and reactive nitrogen species (RNS) (González-Santoyo and Córdoba-Aguilar, 2012).

An additional difference between the IS of insects and ticks is the

IMD pathway. While this pathway is functional in ticks, it lacks some transmembrane peptidoglycan recognition proteins (PGRPs) like imd and dFADD, as well as specific pathways to produce certain AMPs (Chávez et al., 2017). Some transduction signals in ticks are different to those found in insects (e.g., *Drosophila*), resulting in a higher bacterial load in species like *Anaplasma phagocytophilum* (Severo et al., 2013). A loss of genes associated to the IMD pathway has been reported in other HBFA species, like *P. humanus humanus.* Furthermore, the genes for this pathway were not found in the genome of the triatomine *R. prolixus* (Kim et al., 2011; Mesquita et al., 2015). These differences between insect and tick IS could explain why bacterial genera like *Rickettsia* and *Cardinium* are more common, diversified, and abundant in terrestrial chelicerates than in insects (Weinert et al., 2015).

Considering the intimate interaction between PS and arthropod hosts, it might be thought that the IS should not be activated by this bacterial group. However, this is not the case in some HBFA species, as well as in other symbiotic PS-arthropod relationships. For instance, the bacteriocytes that lodge *W. glossinidia* in tse-tse flies express the negative regulator PGRP-LB of the IMD pathway, which prevents AMP synthesis. This indicates that *W. glossinidia* is still recognized as a foreign agent (Wang et al., 2009). For those HBFA species that host *Wolbachia* as a PS, like bedbugs and tse-tse flies, *Wolbachia* would be escaping the humoral and cellular immune responses (Sicard et al., 2014).

In contrast, when *Wolbachia* is located extracellularly in the host, either in tissues or the hemolymph, as reported in many HBFA species analyzed here, its recognition would not be via “traditional” PAMPs like lipopolysaccharides, peptidoglycans, or β-1,3-glucans, which are characteristic of Gram-negative bacteria, Gram-positive bacteria, and fungi, respectively. In fact, very particular proteins, called *Wolbachia* surface proteins (WSPs), have been identified (Sicard et al., 2014).

On this regard, there is some speculation that WSPs could be less reactive in the hosts or even have immunosuppressive effects (Sicard et al., 2014). For instance, in wild populations of *Ae. aegypti*, which are not naturally infected by *Wolbachia*, a higher expression of immunity-related genes has been observed in experimental *Wolbachia* infections (Blagrove et al., 2012). Additionally, an efficient camouflage strategy that may prevent its recognition by the host IS has been observed in *Wolbachia* when it is acting as an endosymbiont (Sicard et al., 2014). It is believed that *Wolbachia* could be sequestrating lipids from the host and producing a protective membrane. This strategy could protect *Wolbachia* from cytoplasmic immune mechanisms like the production of AMPs, ROS, phagolysosomes, apoptosis, and autophagic effectors (Sicard et al., 2014).

Another interesting example is the genus *Rickettsia*. Bacteria of this genus have been classified as infective, although they have been proved to move among cells and tissues in the host (Gottlieb et al., 2012). Once inside the cells of the host, bacteria manipulate cytoskeletal proteins, inducing the formation of an actin tail related to cell motility and virulence (Gottlieb et al., 2012). *Rickettsia* has been proved to activate the expression of the AMP Defensin-1 in the tick species *Dermacentor variabilis* Say (Kopáček et al., 2010). In another tick species, *Dermacentor andersoni* Koch, *Rickettsia peacockii* can be highly resistant to various AMPs (Baldridge et al., 2005). The SS *Sodalis* sp., found in tse-tse flies, is another species highly resistant to the effects of AMPs (Diptericin) (Hao et al., 2001). *Sodalis* also expresses an extracellular variant of the outer membrane protein named OmpA, which prevents virulence to the host (Weiss et al., 2008).

The digestive tract is recognized as a key region in the immune homeostasis of all animals, including HBFA species (Engel and Moran, 2013). In the latter group, the gut could play an even more decisive role, since many parasite and pathogen species enter the host by the oral route, and their life cycle is completed in the host digestive system. As mentioned above, there is strong evidence that the IS in arthropods would always react to symbiont bacteria, even to PS or SS species that were in the gut. The most widely accepted model of this tripartite relationship indicates that:

(a) Symbiont bacteria in the gut would be modulating some pathways in the host IS. Most mechanisms of the immune response in the gut of insects are determined by the IMD pathway, and upon its activation, AMPs and ROS will be produced via the dual oxidase (DUOX)-associated transmembrane system (Engel and Moran, 2013). In ticks, the JAK/STAT pathway is also taking part in the immune response in the gut, although it differs from the IS of insects in the lack of a homologue of the signaling cytokine Unpaired (Upd), which in time activates the Dome receptor (Chávez et al., 2017). On the other hand, lysozyme expression, the PO cascade, and RNS production are found in the digestive tract of triatomines (Flores-Villegas et al., 2015). Although information on the immune mechanisms operating in bedbug and louse gut is scarce, it is known that genes coding for the AMPs Defensin-1 and Defensin-2, as well as for prophenoloxidase (PO zymogen) and PGRP, are expressed when challenged by bacteria, while those genes encoding for the DUOX system and lysozymes are not expressed (Kim et al., 2012). The main gut immune mechanisms in hematophagous dipterans feeding from humans are determined by the IMD, Toll, and JAK/STAT pathways, and by the DUOX system; however, the PO/melanization system can also be expressed (Van Tol and Dimopoulos, 2016).

(b) Once the host IS has been modulated, some bacterial species or certain population densities could be established as determined by the species, sex, and development stage of the HBFA. Besides the characteristic PS and SS species and those shared by all HBFA species, a third important of SS cluster, which is composed by species that colonize the digestive tract; this cluster is formed by *Pseudomonas*, *Acinetobacter*, *Enterobacter,* and *Enterococcus* (Figs. 1 and 3, Table 2). Herein, we noted that they are dominant over the number of HBFA species infecting Diptera, triatomines and ticks (Azambuja et al., 2005; Da Mota et al., 2012; Geiger et al., 2014; Gumiel et al., 2015; Narasimhan and Fikrig, 2015; Van Tol and Dimopoulus, 2016; Vallejo et al., 2009; Fraihi et al., 2017) (Table 2). Further groups of SS reported in several species of HBFA, but in a lower proportion than those listed above, are *Serratia*, *Sphingobacterium*, *Stenotrophomonas*, *Bacillus* and *Staphylococcus* (Da Mota et al., 2012; Fraihi et al., 2017; Geiger et al., 2014; Gumiel et al., 2015; Narasimhan and Fikrig, 2015; Van Tol and Dimopoulus, 2016; Vallejo et al., 2009) (Figs. 1 and 3, Table 2). In HBFA, a selection of strains with hemolytic activity in genera like *Serratia* and *Enterobacter* has been observed; this selection would explain their permanence and evolution in some HBFA groups (Gaio et al., 2011).

(c) Various interactions occur between microbiota and pathogen and/or parasite species in the gut of HBFA. Two forms of competition have been described in HBFA: interference competition and what has been termed as apparent competition (Gerardo and Parker, 2014). In the former case, there is a direct interaction among bacteria and the pathogen/parasite, and because of such interaction bacteria release toxins that affect the other partner. The best-studied case of this type of competition in HBFA is a *Serratia* strain isolated from the triatomine *R. prolixus*, which produces prodigiosin, a pigment that can lyse *T. cruzi* (Azambuja et al., 2004). Another *Serratia* strain has been reported to exhibit inhibitory effects against sporangia and sexual stages of *Plasmodium* (Azambuja et al., 2005). Yet another *S. marcescens* strain was isolated from *Lutzomyia longipalpis* Lutz and Neiva sand flies, which prevents the establishment of *Leishmania mexicana* via ROS production (Sant’Anna et al., 2014). In the second type of interaction, the symbiont bacterial species is stimulating some immune pathway, which in turn is indirectly detrimental for the pathogen or parasite species (Gerardo and Parker, 2014). In the field of invertebrate immunology, this phenomenon has been called immune facilitation or improved protection, and it should be distinguished from immune priming, where the immune response, pathogen or parasite removal, and or improvement in host survival are more intense in a second challenge, but such more intense response is specific against the pathogen or parasite previously administered or put in contact with the host (Kurtz, 2005; Contreras-Garduño et al., 2016). It has been observed that the native bacterial

microbiota regulates the basal immune response in mosquitoes of the genus *Anopheles* and it also controls *Plasmodium* populations, being the IMD and Toll pathways the main immune mechanisms for this. Additionally, it is known that both *Plasmodium* and the bacterial microbiota share genes of the host immune response that code for certain PRRs (mainly PGRP-LC), effectors, and regulators of the immune response mediated by phagocytosis, lysis, and melanization (Van Tol and Dimopoulos, 2016).

A less-well studied phenomenon that is present at least in hematophagous dipterans feeding on humans is the facilitation of parasite establishment and development by some bacterial symbiont species. The best documented example is the interaction between *Sodalis* and tse-tse flies. As mentioned above, *Sodalis* is regarded as a SS in tse-tse flies. Nevertheless, populations of *G. morsitans* infected by *Sodalis* have been reported as more susceptible to infection by *T. brucei* than *Glossina austeni* Newstead populations with low *Sodalis* infection rates (Farikou et al., 2010). This could be a subsidiary result of lectin inhibition in the digestive tract of tse-tse flies. As recently reported, adding antibiotics to the diet of *L. longipalpis* sandflies – thus removing some bacterial species or decreasing their population in the gut affected the replication and development of the metacyclic form of *Leishmania infantum* (Kelly et al., 2017). However, the authors study failed to determine exactly which bacteria genera had that function. The IMD pathway in the mosquito *Culex tarsalis* Coquillet was suppressed by experimental infection with the *Wolbachia* wAlbB strain, which facilitated infections by the West Nile virus (Dodson et al., 2014). Although various *Serratia* strains proved to be antagonistic to *Trypanosoma* and *Leishmania*, their effects on viruses were mixed. *Serratia odorifera* was found to facilitate the infection by dengue virus serotype 2 in the mosquito *Ae. aegypti* (Apte-Deshpande et al., 2012).

8. Symbiont bacteria from HBFA: medical and ecological implications

Microbiota analysis in HBFA groups shows that there is a central group of symbiont bacteria, generally PS, which have developed and evolved mutualistic relationships with their hosts. Their functions are generally associated to metabolic and/or reproductive aspects (Vavre and Braig, 2012; Douglas, 2015). A second important group of symbiont bacteria in hematophagous arthropods' microbiota, which changes dynamically in time and space, is formed by SS species, which usually colonize the digestive tract. This bacterial community is greatly determined by environmental aspects, mainly those related to feeding habits and the geographic region where species are distributed (Engel and Moran, 2013; Geiger et al., 2013).

The number of species in the SS group has grown in recent years. There could be various reasons, not mutually exclusive, behind this observation. Firstly, better molecular techniques are available now, which allow for more complete analyses of animal and plant microbiotas and microbiomes (Douglas, 2015; Finney et al., 2015).

Secondly, it is known that several HBFA species have a wide geographic distribution, and new data on HBFA bacterial communities have been gathered in recent years from places where no previous data existed (see, for instance, recent cases of *Rickettsia* in *Anopheles, Mansonia,* and *Culex* in Africa and China; *Spiroplasma* in various species of tse-tse flies; *Rickettsia*, *Spiroplasma,* and *Wolbachia* in phlebotomine mosquitoes in South America and China) (Doudoumis et al., 2017; Li et al., 2016; Monteiro et al., 2016; Zhang et al., 2016).

Thirdly, and in relation with the previous factor, are the consequences of world-scale problems like large human migrations and the expansion of areas designated for animal farming. This has increased the interactions among wild animals, farm animals, and even pet animals, and their respective hematophagous arthropod species (Léger et al., 2013; Benelli et al., 2018). As a result, some HBFA and HA species are feeding indistinctly from humans and some species of wild or domestic vertebrates, thus promoting an exchange of bacterial species (Thomson, 2014).

Finally, another factor that is contributing to widen the geographic distribution of HBFA species is global warming (Gullan and Cranston, 2014; Thomson, 2014). Various models on vector insect populations predict the following effects: a decrease in generational times, an increase in population growth rates, a decrease in pathogen incubation times, and increase in the length of periods in which transmission occurs (Gullan and Cranston, 2014). Among hematophagous arthropod species, we know that the natural distribution ranges of *Ae. aegypti* and *Ae. albopictus* have expanded, and currently both mosquito species are found in all continents, including North America and Europe (Kraemer et al., 2015). Several tick species have also extended their distribution ranges, and it is estimated that some of them will continue expanding their distribution in the next few years (Léger et al., 2013).

As previously mentioned, it is complicate to foresee all the possible consequences of the entry and establishment of new bacterial species in the microbiota of each HFBA species. Such difficulty arises from the complex network of interactions among the resident microbiota, the arthropod IS, and the new bacterial species entering the host. However, because of the issues discussed above, all possible results of the entry of new bacterial species or strain could impact on human health.

A first consequence would be the transmission of bacteria to human populations. In recent decades, ticks might have been the HFBA group with the highest number of pathogenic species for humans (Vayssier-Taussat et al., 2015; Benelli et al., 2016a, 2017). Sixteen genera had previously been identified as causative agents of disease (*Rickettsia*, *Anaplasma*, *Ehrlichia*, *Coxiella*, *Borrelia*, and *Francisella*) (Vayssier-Taussat et al., 2015; Benelli et al., 2016a, 2017), and species like *Neoerhlichia mikurensis* and *Borrelia miyamotoi* were recently added to the list (Platonov et al., 2011; von Loewenich et al., 2010). *Arsenophonus* and *Midichloria*, both genera of endosymbiont bacteria, have also been reported as possible human pathogens, even though there is not strong evidence of it (Bonnet et al., 2017).

Several *Rickettsia* strains that cause the Rocky Mountain spotted fever have been found in mosquitoes of the genera *Aedes*, *Anopheles*, *Mansonia,* and *Culex* in various African and Asian regions. Additionally, at least *An. gambiae* has been demonstrated to transmit *R. felis* to mice (Dieme et al., 2015; Zhang et al., 2016).

Opposite is the case, at least with respect to human transmission, of *Cimex lectularius*. Even though *C. lectularius* has been reported to host until 21 pathogen species (Delaunay et al., 2011), no evidence exists to date of the transmission of such species to humans (Delaunay et al., 2011; Lai et al., 2016). Possible causes of this phenomenon are related to the peculiar reproduction method of these insects. Males mate females through traumatic inseminations, entering their aedeagus in several body sections of females and causing different types of damage (Stutt and Siva-Jothy, 2001). Under constant mechanic damage, the IS could be constantly activated in females, while a high bacteriolytic activity has been reported in ejaculates and in hemolymph in males (Delaunay et al., 2011; Otti et al., 2009). Bedbug saliva contains several AMPs, and it could be removing bacteria when feeding (Lai et al., 2016).

A second consequence is related to the different species or strains found in or entering each region, which affect the ecology and evolution of each HFBA species in the planet. Several strains that produce bioactive compounds or secondary metabolites have been recently isolated, which could be useful to control various pathogens or parasites. Among the most representative examples of bacterial production of compounds are several *Serratia* strains isolated from culicid mosquitoes and triatomines. These *Serratia* strains produce the pigment prodigiosin, which has proved to inhibit *T. cruzi* (Azambuja et al., 2004), *P. falciparum* (Lazaro et al.,2002), and *Leishmania* sp. (Moraes et al., 2008) both *in vitro* and *in vivo*. An *Enterobacter* strain isolated from *An. gambiae* was proved to inhibit *P. falciparum* through ROS production (Cirimotich et al., 2011). Although Actinobacteria are not widely distributed in HFBA, the group is interesting as a source of

compounds like antibiotics, and its study could be important, particularly in species like triatomines, whose microbiota is highly dependent on environmental elements, mainly on the soil, where this type of bacteria is abundant (Flórez et al., 2015; Gumiel et al., 2015). At least 37 secondary metabolites with potential antibacterial properties were recently found in a metagenomic analysis of bacteria from the phlebotomine fly *L. longipalpis* and from *Triatoma* sp. (Tobias, 2016). As previously discussed, the capacity of non-native *Wolbachia* strains to activate various immune pathways in mosquitoes. When species of *Anopheles* and *Aedes* were transinfected with different strains of *Wolbachia*, the infection and/or transmission of *Plasmodium* species or viruses were blocked and/or impaired (Bartholomay and Michel, 2018; Van Tol and Dimopoulos, 2016). The bacteria *Rhodococcus rhodnii,* which is found in triatominae, has been genetically modified to express cecropin A, a peptide with anti-trypanosome activity (Durvasala et al., 2003). *Asaia*, a common genus in hematophagous dipterans feeding from humans, has also been genetically modified to proliferate when *Plasmodium* enters the body of *Anopheles* sp. and stimulate the insect IS, mainly by activating AMP-producing pathways (Capone et al., 2013).

9. Conclusions and challenges for future research

As outlined in the present review, we have just started to understand the role of bacterial symbiosis as one of the main axes in animal evolution. Among invertebrates, arthropods are the group where the study of animal-bacteria symbiosis begun, and it may be the one where the interaction mechanisms and the effects of each party on each other are best-known. It is very likely that arthropods could evolve in all the feeding forms that we currently know, including hematophagy, thanks to this symbiosis. We are in a crucial moment of human history, when the geographic distribution of various hematophagous arthropod species has been modified by world-scale phenomena like global warming and the high indices of human migration, having therefore that diseases transmitted by these vectors are reaching regions of the planet where they had never been reported until some decades ago, like the recent reports on the Zika and chikungunya viruses.

Our challenge in the next few years will be to analyze how these events affect the microbiota of hematophagous arthropods, including the entry of new species and strains of bacterial symbionts, as well as the removal of others, in the complex microecological network being developed within these arthropods, and how they could affect the transmission of pathogens and parasites for which HAH are vectors, giving them even the capacity to transmit new pathogenic strains and species.

Conflict ofinterests

The Authors declare no competing interests.

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